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SEX-LIMITED INHERITANCE IN POULTRY

C. B. DAVENPORT

From the Station for Experimental Evolution, Carnegie Institution of Washington

EIGHT FIGURES (COLORED PLATES)

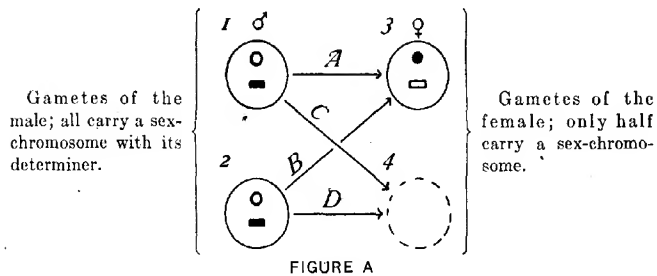
1. SCOPE OF THE PAPER

The problem of sex-limited inheritance has been studied by two converging lines of attack. On the one hand, Doncaster and Raynor ('06), Punnett, Bateson and his pupils in England, and Spillman, Pearl, Morgan and others in this country have used the methods of the experimental breeder; on the other hand, McClung, Stevens and Wilson have used the method of cytological study. The results gained constitute one of the greatest advances made in biology during the present decade, if not in the history of science. The details of the interpretation of the facts have been modified from time to time as light has been thrown upon them from new angles but we seem now to have reached a formula that is satisfactory in its simplicity and is in accord equally with the data of cytology and of breeding. I judge it worth while to take the occasion of the presentation of certain new data to review briefly the earlier experiments on sex-limited inheritance in poultry and to show how the new and simpler formula accounts for the observed facts quite as well as the various interpretations of the respective authors.

2. THE FORMULA

The formula that I shall adopt and apply is that which Wilson has proposed ('11, '11 a) as a consequence of his cytological studies; and which Morgan also, has reached ('10, '11, '11 a) through his experimental work in breeding flies. In its application to poultry, in the form herein adopted, it is that sex-limited characters have their determiners located in the sex-chromosome. This

is not to state that all of the elements out of which such characters arise are located there but that something that plays a predominating rôle in the development of such characters is located there. The male fowl is positively homozygous, or, as I prefer to call it, *duplex*, in respect to the sex determiner just because it has, probably, two sex-chromosomes or their equivalent in the somatic cell. Consequently each of its sperm-cells contains a sex-chromosome and, hence, a determiner for each independent sex-limited character. The female fowl, on the other hand, is heterozygous, or *simplex*, in respect to the sex-determiner just



because it has, probably, one sex-chromosome or its equivalent to the cell. Consequently, only half of the matured eggs of a hen have a sex-chromosome and, hence, a determiner for each independent sex-limited character. The other eggs lack the determiner for any sex-limited character.¹

The consequences of the theory outlined above may best be shown graphically and this I have attempted in fig. A. The circles represent sex-chromosomes and the included figures are

¹ It must be admitted that, while the parallelism of cytological and experimental results has been shown in several cases, yet in the only published observations on the spermatogenesis of the fowl (Guyer, '09, p. 579) it is concluded that in the cell division that gives rise to the definitive sperm cells "the odd chromosome passes undivided to one pole in the vast majority of cases." It must, however, be considered that the chromosomes of the testis of the fowl are particularly crowded and hard to disentangle and one is justified in awaiting a confirmation of Guyer's results before attempting to bring them into harmony with the theory here adopted or rejecting a theory which explains practically all the results of experimental breeding of sex-limited characters.

symbolical of the presence of two sex-limited characters. The presence of a determiner for a sex-limited character is indicated by a solid symbol; the absence of the determiner by a hollow symbol. The absence of a sex-chromosome is represented by a dotted circle without included determiner.

Let 1 and 2 represent the sex-chromosomes of the sperm cells of one race, present and alike in all the sperm. Let 3 represent from another race, one of the eggs that contains the sex-chromosome and 4 one of the eggs that is without it. Let the two kinds of eggs be equally numerous and equally apt to be fertilized. By the theory the union of two germ-cells that both contain sex-chromosomes determines a male; the fertilization of an egg that lacks a sex-chromosome by any of the sperms determines a female. It is clear that, in the male progeny, determiners for sex-limited characters come from both germ-plasms and the sons show the dominant sex-limited characters of both races; on the other hand the daughters show the sex-limited characters of their sires only. It is easy to see that in a reciprocal cross the sons will show the same characters as before but the daughters, inheriting their sex-limited characters solely from their fathers, will be different because they have different fathers.

3. SEX-LIMITED CHARACTERS HERETOFORE DESCRIBED OF THE TYPE SHOWN BY POULTRY

1. The first case of sex-limited inheritance in the modern sense that was worked out by modern methods of analysis and with an approximation to the modern interpretation was, as is well known, that of the English current moth, *Abraxis grossulariata*, and its variety, lacticolor. This brilliant achievement of Doncaster and Raynor ('06) marks an epoch. According to our present interpretation this case is simply one in which the variety (lacticolor) has arisen by the dropping out of a factor, *G*, possessed by the *grossulariata*, so that lacticolor may be represented by *g* (table 1).

The results of Doncaster and Raynor agree closely with expectation as indicated at the right of table 1, and sustain the hypothesis that in the female the sex-determiner is simplex.

TABLE 1

	σ^{σ}		$\varphi \varphi$	REMARKS
1	grossul.	\times	lacticol.	
Gametes	$\begin{cases} G \\ g \end{cases}$		$\begin{cases} G \\ g \end{cases}$	
F ₁	Gg		G	Both σ^{σ} and $\varphi \varphi$ hybrids are grossul.
2	F ₁ (grossul. \times lacticol.) \times F ₁ (grossul. \times lacticol.)			
Gametes	$\begin{cases} G \\ g \end{cases}$		$\begin{cases} G \\ g \end{cases}$	
F ₂	$\begin{cases} GG \\ Gg \\ gg \end{cases}$		$\begin{cases} G \\ g \end{cases}$	All $\sigma^{\sigma} \sigma^{\sigma}$ grossul.; 50 per cent $\varphi \varphi$ grossul. 50 per cent $\varphi \varphi$ lacticol.
3	F ₁ (grossul. \times lacticol.) \times		lacticol. φ	
Gametes	$\begin{cases} G \\ g \end{cases}$		$\begin{cases} G \\ g \end{cases}$	
Offspring	$\begin{cases} Gg \\ gg \end{cases}$		$\begin{cases} GG \\ g \end{cases}$	50 per cent $\sigma^{\sigma} \sigma^{\sigma}$ and 50 per cent $\varphi \varphi$ grossul.; the others lacticol.
	lacticol. σ^{σ}	\times	F (grossul. \times lacticol.) φ	
Gametes	$\begin{cases} g \\ Gg \end{cases}$		$\begin{cases} G \\ g \end{cases}$	
Offspring	$\begin{cases} Gg \\ Gg \end{cases}$		$\begin{cases} g \\ g \end{cases}$	All $\sigma^{\sigma} \sigma^{\sigma}$ grossul. all $\varphi \varphi$ lacticol.

2. The case of the cinnamon canary was worked out by Durham and Marryat ('09) confirming and greatly extending the fancier's knowledge of breeding in this race. As is well known, we have in the cinnamon race a dilute black pigment in the plumage and in the iris giving the juvenile 'pink' eye. The green and the yellow canaries have, on the other hand, a black iris, which we may designate by *B*. The pigment condition in the cinnamon is represented by *b*. The results to be expected from various matings are given in table 2. The actual results obtained from the given matings agree closely with expectation.

TABLE 2

Unions of sex-limited determiners for black iris pigmentation in pink-eyed × black-eyed canary matings.

	♂♂	×	♀♀	REMARKS
1	black-eyed		pink-eyed	
Gametes	$\begin{cases} B \\ B \end{cases}$		b	
F ₁	Bb		Bb	All hybrids of both sexes are black-eyed
2	pink-eyed		black-eyed	
Gametes	$\begin{cases} b \\ b \end{cases}$		B	
F ₁	Bb		b	All males are black-eyed; all females pink-eyed
3	F ₁ black-eyed		pink-eyed	
Gametes	$\begin{cases} B \\ b \end{cases}$		b	
Offspring	$\begin{cases} Bb \\ bb \end{cases}$		$\begin{matrix} B \\ b \end{matrix}$	Half of the ♂♂ and half of the ♀♀ black-eyed; the others pink-eyed
4 F	F ₁ black-eyed		black-eyed	
Gametes	$\begin{cases} B \\ b \end{cases}$		B	
Offspring	$\begin{cases} BB \\ Bb \end{cases}$		$\begin{matrix} B \\ b \end{matrix}$	All males are black-eyed; 50 per cent ♀♀ black-eyed; others pink-eyed

3. The case of the melanogenesis in the skin of the Silky fowl was worked out by Punnett in connection with Bateson ('09) in a series of experiments. The interpretation that they gave to their results is complicated; that afforded by the new theory affords a simpler explanation. The unpigmented skin appears to have an inhibitor (I) which the black skin of the Silky lacks (i).

Table 3 gives the various matings and the proportions to be expected. On account of the facts that (a) pigment develops slowly and most of the offspring had to be described early and (b) that more than one factor may be involved in the production of pigment, the relation of actual to expected was not always very close.

TABLE 3

	♂♂	•	♀♀	REMARKS
1	Brown Leghorn	×	Silky	
Gametes	$\begin{cases} I \\ i \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
F ₁	Ii		I	In all hybrids melanogenesis is inhibited
2	Silky	×	B. Leghorn	
Gametes	$\begin{cases} i \\ i \end{cases}$		I	
F ₁	Ii		i	In all males melanogenesis is (partly) inhibited; but in ♀♀ not
3	F ₁ (Silky ♂ × Leghorn ♂) × F ₁ (Silky ♂ × Leghorn ♀)			
Gametes	$\begin{cases} I \\ i \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
F ₂	$\begin{cases} Ii \\ ii \end{cases}$		$\begin{cases} I \\ i \end{cases}$	In both sexes, approximately half have and half lack the melanogenetic inhibitor
4	F ₁ (Leghorn ♂ × Silky ♀) × F ₁ (Leghorn ♂ × Silky ♀)			
Gametes	$\begin{cases} I \\ i \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
F ₂	$\begin{cases} II \\ Ii \end{cases}$		$\begin{cases} I \\ i \end{cases}$	No fully pigmented male birds expected (though some found) as young; and ♀♀ half pigmented, half unpigmented

TABLE 3—Continued

5	$F_1(\text{Leghorn} \times \text{Silky})$	\times	Brown Leghorn	
Gametes	$\begin{cases} I \\ i \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
Offspring	$\begin{cases} II \\ Ii \end{cases}$		$\begin{cases} I \\ i \end{cases}$	No fully pigmented $\sigma^7 \sigma^7$; half of the $\varnothing \varnothing$ pigmented
6	Brown Leghorn	\times	$F_1(\text{Leghorn } \sigma^7 \times \text{Silky } \varnothing)$	
Gametes	$\begin{cases} I \\ I \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
Offspring	$\begin{cases} II \\ Ii \end{cases}$		$\begin{cases} I \\ i \end{cases}$	In all birds deep pigmentation is inhibited
7	$F_1(\text{Leghorn} \times \text{Silky})$	\times	$F_1(\text{Silky } \sigma^7 \times \text{Leghorn } \varnothing)$	
Gametes	$\begin{cases} I \\ i \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
Offspring	$\begin{cases} Ii \\ ii \end{cases}$		$\begin{cases} I \\ i \end{cases}$	In both sexes equal numbers of uninhibited and inhibited pigmentation
	Silky	\times	$F_1(\text{Leghorn } \sigma^7 \times \text{Silky } \varnothing)$	
Gametes	$\begin{cases} i \\ i \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
Offspring	$\begin{cases} Ii \\ ii \end{cases}$		$\begin{cases} I \\ i \end{cases}$	In all $\sigma^7 \sigma^7$ pigmentation only partly inhibited; all $\varnothing \varnothing$, deeply pigmented
9	Silky	\times	$F_1(\text{Silky } \sigma^7 \times \text{Leghorn } \varnothing)$	
Gametes	$\begin{cases} i \\ i \end{cases}$		$\begin{cases} I \\ i \end{cases}$	
Offspring	$\begin{cases} Ii \\ ii \end{cases}$		$\begin{cases} I \\ i \end{cases}$	All offspring fully pigmented

4. Barring in Plymouth Rock poultry follows a similar law to Silky pigmentation. Spillman ('08) first pointed this out for the Barred Rock \times Indian Game cross and his results were confirmed by Pearl and Surface ('10, '10 a, '10 b). Goodale ('09

and '11) has studied sex-limited barring in Barred Rock \times Buff Rock and Barred Rock \times Brown Leghorn crosses. In general the results of these matings, on the new hypothesis, are as indicated in table 4, where B stands for the barring factor and b for its absence.

5. That the black and red markings of the Brown Leghorn and the gray of the White Wyandotte are also sex-limited is maintained by Sturtevant (11) in a brief notice. When the Brown Leghorn was used as the father the eight daughters were all brown; but in the reciprocal cross they (all three) were gray; the male offspring were gray in both crosses.

TABLE 4
Inheritance of barring in barred \times non-barred matings

	σ^{σ}	\times	φ^{φ}	REMARKS
1	barred	\times	non-barred	
Gametes	$\begin{cases} B \\ b \end{cases}$		$\begin{cases} B \\ b \end{cases}$	
F_1	Bb		B	All birds are barred
2	non-barred	\times	barred	
Gametes	$\begin{cases} b \\ b \end{cases}$		$\begin{cases} B \\ b \end{cases}$	
F_1	Bb		b	All σ^{σ} barred; all φ^{φ} non-barred
3	F_1 (barred $\sigma^{\sigma} \times$ non-barred φ^{φ})	\times	F_1 (barred $\sigma^{\sigma} \times$ non-barred φ^{φ})	
Gametes	$\begin{cases} B \\ b \end{cases}$		$\begin{cases} B \\ b \end{cases}$	
F_2	$\begin{cases} BB \\ Bb \\ bb \end{cases}$		$\begin{cases} B \\ b \end{cases}$	All σ^{σ} barred; females, 50 per cent barred; 50 per cent non-barred
4	F_1 (non-barred $\sigma^{\sigma} \times$ barred φ^{φ})	\times	F_1 (non-barred $\sigma^{\sigma} \times$ barred φ^{φ})	
Gametes	$\begin{cases} B \\ b \\ Bb \\ bb \end{cases}$		$\begin{cases} b \\ B \\ b \end{cases}$	
				50 per cent of σ^{σ} and 50 per cent of φ^{φ} non-barred; the others barred

6. The Black-red Game Bantam has the same color as the Brown Leghorn. Hagedoorn ('09) crossed it with a Brown-red Game, which differs from the Leghorn chiefly in that, in the male, the black breast feathers are laced with brown and the females are all black except for the brown lacing of the nest feathers. But a more important difference is seen in the chicks; for in the Black-red Game the chicks are striped while in the Brown-red Game they are black in both sexes. Striping, S , is dominant over its absence, s (table 5).

As a matter of fact Hagedoorn did not get the expected result in mating 3, which may be due to the fact that he had too few offspring. He states that all young females were like the mothers and all males *tested* were heterozygous like the father.

7. When Brown Leghorn is crossed with White Plymouth Rock there is an apparently more complicated sex-limited inheritance, since the White Rock carries the barring factor, B , but lacks the color factor, C (Goodale, '10). Calling L the factor that determines the Brown Leghorn coloration and l its absence; C the color-factor (which is not sex-limited) and c its absence, the

TABLE 5

	♂♂	♀♀	REMARKS
1	black-red	× brown-red	
Gametes	$\begin{cases} S \\ s \end{cases}$	$\begin{cases} S \\ s \end{cases}$	
F ₁	Ss	S	All chicks are striped
2	Brown-red	× black-red	
Gametes	$\begin{cases} s \\ S \end{cases}$	$\begin{cases} S \\ s \end{cases}$	
F ₁	Ss		All males striped and black-red; all ♀♀ un-striped and brown-red
3	F ₁ (black-red ♂ × brown-red ♀) × black-red ♀		
Gametes	$\begin{cases} S \\ s \end{cases}$	$\begin{cases} S \\ s \end{cases}$	
F ₁	SS	S	
	Ss	s	All males and 50 per cent females striped; others unstriped

results of this mating are shown in table 6. The numbers of offspring obtained were small. To the right is given the approximate numerical result.

4. THE NEW CASE OF A SEX-LIMITED CHARACTER IN POULTRY

1. Material employed

In my experiments I used poultry belonging to the Brown Leghorn and the Dark Brahma races—two races that have very dissimilar plumage colorations and exhibit a sex-dimorphism in pattern and coloration. They are, consequently, well adapted to testing again the behavior of sex-limited characters in fowl. The points of difference considered in the two races are indicated in table 7 both for the males and the females.

TABLE 6

	♂♂	♀♀	REMARKS
1	White Rock	× Brown Leghorn	
Gametes	$\begin{cases} Bc \\ Bc \end{cases}$	$\begin{cases} LC \\ -C \end{cases}$	
F ₁	BcLC	BCc	Both sexes barred; the males, only splashed with Brown Leghorn
2	Brown Leghorn	× White Rock	
Gametes	$\begin{cases} L \\ l \end{cases}$	B	
F ₁	BL	L	The males only barred; the females either black with orange-laced hackle or approximately Brown Leghorn
3	White Rock	× F ₁ (White Rock ♂ Brown Leghorn ♀)	
Gametes	$\begin{cases} Bc \\ Bc \end{cases}$	$\begin{cases} BC \text{ or } Bc \\ -c \text{ or } -C \end{cases}$	
Offspring	$\begin{cases} BBcc \\ BBCc \end{cases}$	$\begin{cases} Bcc \\ BCc \end{cases}$	Half ♂♂ white; half barred; ♀♀ half white, half barred

TABLE 7
Sex-dimorphic racial characteristics

CHARACTERS	BROWN LEGHORN	DARK BRAHMA
<i>A: Male (Figs. 1 and 2)</i>		
Lacing (marginal coloration) of hackle and saddle.....	Red	White (dominant)
Upper wing coverts (wing bow)	Red	White, trace of red in middle zone
<i>B. Female (Figs. 3 and 4)</i>		
Lacing of hackle.....	Golden yellow	Silver white
Back and upper wing.....	Light brown, finely stippled with darker	Gray, with three con- centric loops of black (penciling)

2. Matings and results

The F₁ generation. In 1910 I mated (Pen 1009) a Brown Leghorn cock² (No. 14123) to various Dark Brahma hens that had been bred by me and were descended from Nos. 121 ♀ and 122 ♂ described by me in a former publication (Davenport, '06). The following mothers produced offspring that grew to maturity so that their permanent plumage could be described: Nos. 5835, 7549, 7859, 7869, 8001, 11160. Moreover, I mated in Pen 1015 a Dark Brahma cock (No. 11161) to a Brown Leghorn hen of the same origin as the cock of Pen 1009. About fifty-four chicks were reared from these two pens and their adult plumage color studied. The distribution of plumage colors in the two sexes and the two sets of experiments is set forth in table 8.

Table 8 gives a definite answer to the question of the method of inheritance of the characteristics considered. In the reciprocal crosses of Pens 1009 and 1015 the 27 males are all alike; but the females differ according as the Brown Leghorn or the Dark Brahma is used as father and in their hackle color they resemble, in both cases, the father (figs. 5 and 6, 7 and 8).

² Bred by and purchased from H. W. Smith, Islip, Long Island, N. Y.

TABLE 8

Frequency of specified characters in the offspring in the given matings

MATING 1009. F ₁ (BROWN LEGHORN ♂ × DARK BRAHMA ♀)								MATING 1015. F ₁ (DARK BRAHMA × BROWN LEGHORN)
CHARACTERISTICS	MOTHERS' NUMBERS						TOTAL	14129
	5837	7549	7859	7899	8001	11180		
Males with lacing white..	2	9	7	2	2	1	21	2+
Males with lacing red....	0	0	0	0	0	0	0	0
Males with wing bar white	0	0	0	0	0	0	0	0
Males with wing bar red...	2	9	7	2	2	1	21	6
Females with lacing white	0	0	0	0	0	0	0	4
Females with lacing golden	2	7	3	7	2	4	23	0

The male offspring, although all remarkably alike, are like the males of neither of the races involved: for they have the white lacing that is characteristic of the Dark Brahma and the red upper wing coverts that are characteristic of the Brown Leghorn—thus their characteristics are clearly derived from the germ-plasm of *both* parents. Can the same be said of the characteristics of the female offspring? So far as concerns those characters that are not sexually dimorphic such inheritance from both parental germ-plasms is clear, for the pullets of the reciprocal crosses are alike in having pea combs instead of single combs and in having slight in place of heavy booting. Ordinary somatic characters follow the ordinary laws of equivalence in reciprocal crosses.

Even in the sexually dimorphic character of pattern of the feathers of the breast, back, and upper wing there is only a slight difference in the reciprocal crosses. Thus, when the Dark Brahma is taken as father, while the pullets (fig. 8) all have the gray background on the feather correlated with the *white hackle*, the broad concentric loops, on the contrary, shown in fig. 4, are replaced by finer loops which are more or less discontinuous and show a transition to the condition of stippling characteristic of the Brown Leghorn hen. The pattern is quite the same in the pullets (fig. 7) derived from the Brown Leghorn father. The pattern is truly *intermediate*. Also, in both crosses, the amount of red on the wing in the pullets is intermediate (figs. 7, 8) although

the general ground color is determined almost exclusively by the father's germ plasm. The shafting, likewise, is intermediate, inasmuch as it is sometimes absent (though usually present to an intermediate degree) in pullets from a Brown Leghorn sire and is usually absent, but sometimes (as in fig. 7) shows, in pullets from a Dark Brahma sire. Even though we make every allowance for the circumstance that the pullets figured are only about eight months old—an age at which the sharp contrasts of the adults are lacking—still the conclusion can not, I think, be avoided, that not all sex-dimorphic characters are sex-limited.

The F_2 generation. In the spring of the following year (1911) I mated together the (Brown Leghorn σ \times Dark Brahma φ) hybrids in Pens 1112 and 1115; and the (Dark Brahma σ \times Brown Leghorn φ) hybrids were mated in Pen 1126. The results from these two sets of matings are given in table 9.

Expectation in the mating of a male F_1 (Brown Leghorn σ \times Dark Brahma φ) by a female of the same origin may easily be deduced by the formulae used in the earlier part of this paper. Table 10 gives the formula for inheritance of lacing; W , white and w , absence of white.

This formula indicates that half of the father's sperm have and half lack the W factor (inhibitor of formation of red pigment). By hypothesis only half of the eggs have the sex-chromosomes and these eggs all carry absence of white (i.e. golden) hackle. Consequently, white \times golden (= *white*) and no white, or red, \times golden (= *red*) combinations for hackle color should be equally common in the males; actually there are eight white hackles to four red. Though the numbers are small and the accord is proportionately not close yet the absolute departure from equality is small and the result is of the expected order—both types of hackle color occur in approximately the ratio of 1 : 1. Likewise, since, by hypothesis, the sex-limited characters of the pullets are derived solely from the sperm there will be two sorts of pullets, equally numerous, viz., white-laced and golden-laced. Actually these occur in the proportions of 10 to 7. Again the proportions are of the expected order.

C. B. DAVENPORT

TABLE 9
The distribution in the F₂ generation of the sex-limited characters

CHARACTERISTICS	MATING 1112. F ₂ . BROWN LEUCOEN ♂ × DARK BRAHMA ♀					MATING 1115. F ₂ . BROWN LEUCOEN ♂ × DARK BRAHMA ♀					MATING 1126. F ₂ . DARK BRAHMA ♂ × BROWN LEUCOEN ♀				
	MOTHERS' NUMBERS					MOTHERS' NUMBERS					MOTHERS' NUMBERS				
	14751	15019	15554	16174	16298	16840	11712	14766	15429	15432	16108	16437	TOTAL	14803	15536
Males with facing white.....	1	1	0	0	1	1	1	0	1	1	1	0	8	2	0
Males with facing red.....	0	1	0	1	0	2	0	0	0	0	0	0	4	0	0
Males with wing- bar white.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Males with wing- bar red.....	1	2	0	1	1	3*	1	0	1	1	0	1	12	1	0
Females with fac- ing white.....	3	0	1	0	0	1	0	2	1	0	2	0	10	1	1
Females with fac- ing golden.....	1	0	0	1	1	1	0	0	0	1	0	2	7	0	2

* Includes one case with "a little red" on wing bar, doubtless due to imperfect dominance.

TABLE 10

F_1 (Brown Leghorn ♂ × Dark Brahma ♀) × F_1 (Brown Leghorn ♂ × Dark Brahma ♀)

Inheritance of hackle lacing

Gametes	$\begin{cases} W \\ w \end{cases}$	w	
F_2	$\begin{cases} Ww \\ ww \end{cases}$	$\begin{matrix} W \\ w \end{matrix}$	50 per cent of the males with white hackle; 50 per cent with red; same with the females

Returning to the males, the inheritance of the color of the wing-bar has to be considered. By hypothesis, the sperms of the hybrids carry in equal numbers, red-bar and no red (white) bar; while the eggs all carry red-bar. Two kinds of males with the same external appearance are, accordingly, to be expected; namely, duplex and simplex red-bar. All offspring recorded had red wing-bar, but in some cases less strongly developed than in the pure Brown Leghorn race. In all cases, then, the results agree with hypothesis.

The matings of hybrids of the reciprocal cross (viz., Dark Brahma ♂ and Brown Leghorn ♀) yielded few offspring, only two males and four females; but the results are not without interest. By hypothesis, in hybrid males half of the sperms have the determiner for white lacing and the remainder for red lacing; also half of the sperms have the determiner for red wing-bar and half lack it. All hybrid pullets carry the determiner for white lacing in half of their eggs and for white wing-bar in half of their eggs. It follows that of male offspring all should have white lacing, and half should have red and half white wing-bar. Of the female offspring, on the other hand, half should have red and half white lacing. The formulae are as given in table 11. Actually each class is represented in exactly the expected proportions.

TABLE 11

F_1 (Dark Brahma ♂ × Brown Leghorn ♀) × F_1 (Dark Brahma ♂ × Brown Leghorn ♀)

For hackle lacing, W

	♂♂	♀♀	REMARKS
Gametes.....	W w	W	
F_2	WW Ww	W W	Expectation; all ♂♂ and 50 per cent females with white lacing; 50 per cent ♀♀ with red lacing

For wing bar R, evident in males only

Gametes.....	R r	r	
F_2	Rr rr		Expectation; 50 per cent males with red wing bar and 50 per cent with white

CONCLUSIONS

The foregoing observations thus accord with the hypothesis that the male carries two sex-chromosomes and the female one; and that the determiners for certain secondary sex-characters are centered in the sex-chromosomes. We have still to consider the question: Are all sex-limited characters carried in the same chromosome? This does not imply that the sex-limited characters of one variety shall always appear together. This is obviously opposed to the facts, for in the first hybrid generation a white hackle (Dark Brahma) and red wing-bar (Brown Leghorn) appear together; and must always do so when the determiners for these dominant traits are in the same zygote. In F_2 from Brown Leghorn grandfathers two kinds of males are possible on the hypothesis that the two sex-limited determiners are united, as in the same chromosome: namely, with both red hackle and red wing bar (consequently, quite like the pure bred Brown Leghorn) and with red hackle and white wing-bar (the hybrid type); and these,

and they only, were realized. Thus the combination white hackle—white wing-bar (pure Dark Brahma type) did not occur. In F_2 from Dark Brahma grandfathers two kinds of males are possible, viz., those like the pure bred Dark Brahma and hybrids. Of the two males that matured one belonged to the Dark Brahma type and one to the hybrid type but the Brown Leghorn combination did not reappear. Both sets of experiments thus speak strongly for the conclusion that all sex-limited characters are linked together in the sex-chromosome.

Finally, our study throws light on the question whether all secondary sex-characters are sex-limited—in the sense of being carried in the sex-chromosome. For if they are we should expect the female hybrid to inherit such characters from her father's race only. But as we have seen, this is not the case. The details of penciling, stippling, and shafting show clear evidence of blending of conditions from both parental races, in the first hybrid generation. Figs. 7 and 8 show how like the feather pattern is in the pullets of reciprocal crosses. This leads us, provisionally, to classify sex-dimorphic characters into two classes: *a*, characters whose development is controlled primarily by determiners located in the sex-chromosomes and, *b*, characters whose development is specially influenced or modified, probably by secretions of the sex-glands.

January 19, 1912

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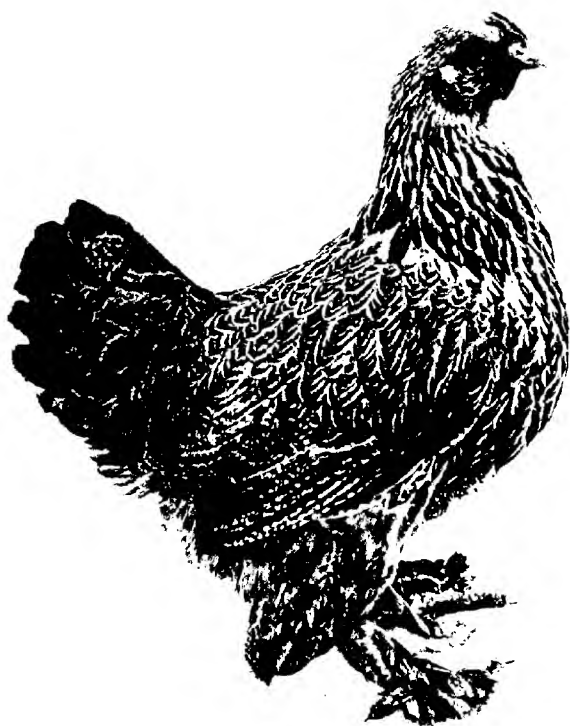
1 Male Brown Leghorn



2 Male Dark Brahma; above and at right a feather from the fifth, the third, and the first row of wing coverts



3 Female Jungle Fowl to show type of coloration of female Brown Leghorn



4 Female Dark Brahma



5 F₁ male hybrid between Dark Brahma ♂ and Brown Laghorn.



6. ♂ male hybrid between Brown Leghorn x and Dark Brahma x



7 F₁ female hybrid between Brown Leghorn ♂ and Dark Brahma ♀



8. F₁ female hybrid between Dark Brahma ♂ and Brown Leghorn ♀

HEREDITY OF BODY COLOR IN DROSOPHILA

T. H. MORGAN

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FOUR COLORED FIGURES—PLATE I

Cultures of the fruit fly, *Drosophila ampelophila*, have given rise to three mutations in the color of the body and wings. The origin of these new types has been briefly described in a preliminary note, and some of the main facts connected with their inheritance have been given there, but the principal data on which the statements rest have been reserved until the present time. The results include 81,070 counts. It may be asked what advantage is there in doing the experiments on so large a scale. Why would not a few cases with suitable tests show the mode of inheritance of the factors involved? The answer is two-fold. The question of the relative viability can only be determined in this way, and for further work with these body colors it is necessary to know what rôle this condition plays in the numerical results. In the second place it seemed worth while to illustrate on a large scale the phenomenon of sex-linked inheritance. It is an impressive fact, for instance, to find in the F_2 generation (out of black female by brown male) 6124 black females, 3015 black males and 2472 brown males. Not a single brown female in 11,000 grandchildren. While in the reciprocal cross there are present 2191 black females, 1987 black males, 1532 brown females and 1448 brown males. Such results cannot fail, I think, to impress those who take a sceptical attitude toward the modern study of heredity.

The black and the yellow mutants arose directly and independently from inbred, normally colored, or gray flies. The brown flies were produced by crossing and extracting. They also arose independently in cultures related to the black flies

but were at first supposed to be a particular kind of yellow fly which was recognized, however, as different from the ordinary yellow and had been maintained in a separate culture. When, however, in the second generation from black and yellow these same flies appeared, their relation to the other colors was apparent. Forms like these, that represent a type due to absences, derivable through combination of primary mutations may be said to arise by permutation.

In making the counts I have been assisted by Miss E. M. Wallace, Miss Eleth Cattell and Mr. C. B. Bridges. In practically all cases I have checked up each count after the separation had been made, so that the results stand for the agreement of two observers. Only in the case of separation of yellows and browns could any disagreement arise, and while I cannot claim for this case that the separation has been exact, I think that it is very nearly so.

Description of the mutants

The color differences between the normal, wild, or gray fly and the mutants is shown by the accompanying plate, figures 1 to 4. The detailed comparisons are as follows:

The wild fly. The upper surface of the thorax is olive yellow, the olive shade being very faint. As the flies get older the color deepens. Some of the wild flies have a black trident and two lateral black streaks on the upper surface of the thorax (not shown in the present case), but many flies do not show this marking. I have made a long series of selection experiments with this marking and have produced one race that never shows the trident, and another race that has a dark well-developed trident. How far the character is a fluctuating one and how far due to genotypic difference need not be discussed here.

The abdomen of the female is banded with lemon yellow and black. In the male there are only two black bands as a rule; the end of the abdomen is black. The legs are colored like the thorax but somewhat lighter. The hairs on the body are black.

The wings are very transparent, blue gray or smoky. The veins appear dark, but under the microscope are seen to be

brownish yellow. There are no dark bands along the sides of the veins. The upper surface of the head of the wild fly, and also of the mutants is colored like the thorax. The under surface of the abdomen is more yellow in the male, than in the female.

The yellow fly. The upper surface of the thorax is yellow ochre in color and lighter than that of the wild fly. It is interesting to observe that the dark marking or shield is always absent from the yellow flies.

The light bands on the abdomen are of the same color as the thorax, i. e., pure yellow ochre, and lighter than those of the wild fly. The dark bands are brown. The legs are the same color as the thorax. The veins of the wings are yellow like the thorax. The interspaces (or background) are of a transparent golden yellow and strongly contrast with the color of the wings of the wild fly. The hairs are brown, instead of black as in the wild fly.

The black fly. The upper surface of the thorax is the same general color as that of the wild fly, but darker in the sense of being browner. The black trident is always present and conspicuous, and the two lateral markings are also conspicuous. The trident is not only well developed, but appears to be longer and narrower than that of the normal. It is one of the most striking features of the black mutant. The light bands of the abdomen are darker than those of the normal, but not so dark as is the thorax. The dark bands are very black. The legs are blacker than the legs of the wild fly, especially the more distal parts. The hairs on the body are black. The veins of the wings are very black. On each side of each vein there is a dark (semitransparent) band. The interspaces between these bands are gray, but darker than the gray of the wing of the wild fly. While the black fly is blacker than the wild fly in nearly all of its parts, so that a heap of them is very dark compared with a heap of the normal flies, the most striking character that distinguishes them from the other types is the dark wings with the dark bands on the sides of the veins.

The brown fly. The upper surface of the thorax is brown. The brown color deepens as the fly gets older, and the color

shown in the figure is that of an older fly. When first hatched the brown is more yellowish in shade, and not so easily distinguished from that of the older yellow flies. Most of the brown flies, especially the older ones, show a brown shield on the thorax, in form like that on the thorax of the black fly. The light bands of the abdomen are light brown and differ therefore from the yellow bands of the yellow fly. The dark bands are brown. The legs also show some brown. The veins of the wings are brown like the thorax. On each side of each vein is a brown band. It is by means of these bands that one can most readily separate under a lens the yellow from the brown fly. The interspaces between the bands are gray-brown, and more transparent than the bands. The hairs are also brown.

In separating the flies into the color groups, (after slightly etherizing) there is never any difficulty in distinguishing the gray from the black and from the yellow, provided they have not just hatched; but the yellow flies and the brown offer greater difficulties, especially when flies of different age are mixed together, and when small flies are also present, due to starving the larvae. I do not feel certain that the separation of these two groups has been always perfect, but the errors are not great enough I think to vitiate seriously the classification. In some cases I have kept the flies alive for several days in order to verify my first separation, and have found occasionally that one or two flies have been put into the wrong group. The errors have been in both directions, and counterbalance to some extent. I have followed the rule of classifying flies as brown only when they were certainly brown, as shown best by the broad brown bands along the veins of the wings, and this has lead, I fear, to the inclusion of a few brown flies in the yellow group. I have also tested my ability to separate these two groups by breeding doubtful flies, which, in general, I would have placed in the yellow category. If the fly in question is a yellow female and is bred to a black male, all the female offspring should be gray (because only the female producing sperm carries the factor for black) and all the males should be yellow. If the fly in question were a brown female, and were bred to a black male,

all the female offspring should be black and all the males brown. In general I have found my separation to be correct. The difficulty has arisen apparently in most cases with heterozygous yellow females that contain only one dose of yellow instead of two, as does the ordinary pure yellow. In such cases two classes of females appear when crossed to black males, namely, gray and black,

In regard to the distribution in the wild fly of the products of the three color factors, that go to produce its color, it is difficult to speak with certainty, but from comparison with corresponding regions in the mutants when one or another of the color factors is absent it appears that the black regions are due to the black factor, but the brown may be present and overlaid by the black. At least it may be said that the black regions in the gray fly and in the black fly are brown in the brown fly, but of course it is possible that when the black develops the brown may not develop, or the black may even be a further stage of development of the brown pigment. The yellow of the wild fly also seems to replace the brown of the brown fly at least when yellow is absent the color of the yellow regions is brown. Possibly, as I have suggested, yellow when present inhibits brown, for otherwise it is difficult to see how the yellow fly should be lighter in color than the brown fly.

The black flies are large and vigorous. There is no difficulty in breeding them or in crossing. The yellows are generally smaller (though not always) and are more delicate. They get stuck very easily to the moist sides of the culture bottles, and, being unable to free themselves, perish. They are more difficult to breed and to cross. The brown flies although generally large are weak. They get stuck to the food and to the walls of the bottles and die. Otherwise they seem healthy. On the whole the mutants are weaker than the normal flies, but the loss of the yellow factor that produces the black flies is less injurious than the loss of the black factor that produces the yellow fly. I have at times thought that the loss of both of the factors produces the weakest fly in the series—the brown fly—but this is difficult to prove.

Formulas

The color of the wild fly appears to be due to the presence of three factors, Black (B), Yellow (Y) and Brown(Br). For brevity this color is spoken of as Gray, which corresponds nearly to the color of the semi-transparent wings. If the black factor (B) is absent (b) the color of the fly is yellow (Y), more especially the wings. The yellow fly is therefore bYBr. Where the factor for yellow (Y) is absent (y) the fly is black, more especially the wings. The black fly is therefore ByBr. When both black and yellow are absent the fly is brown, more especially the wings. The brown fly is, therefore, byBr. The brown fly can always be produced by crossing yellow and black and inbreeding the F₁'s which give by recombination some Browns in the second, F₂, generation. Of course, the same result would follow if both yellow and black were lost from the gray fly at the same time, but this is unlikely since the black and the yellow factors lie in different parts of the hereditary complex.

Of these three color factors that of black is sex-linked; the yellow factor is not sex-linked, and is contained in all gametes both in the male and in the female of gray and yellow flies. One must be careful to observe that while the *factor* for black is sex-linked the black fly, bred to gray does not show sex-linked (sex-limited) inheritance; while the yellow fly bred to normal shows sex-linked inheritance. This will be clear from an examination of the analyses given below.

All possible crosses have been made between these mutants, and, these may now be taken up in order.

Wild (gray) by black

When the female wild flies (Gray) are mated to male black flies all of the offspring are gray. These F₁ gray flies are darker than the wild flies, i. e., they are to some extent intermediate in color between gray and black. It is true that there is much variation in these hybrids, and some flies can not always with certainty be distinguished from wild flies, but most of them are undoubtedly distinctly darker, especially the wings. How this

could occur may not appear clear at first sight, for both the wild gray females and the gray-black hybrids contain two doses of black—the only difference between the two is the absence of one dose of yellow, in the hybrids. If, in the absence of this dose of yellow, the black has a better chance to show itself more positively, we can account for the intermediate character of the hybrid. If such is the case, the yellow factor is to some extent a partial inhibitor of black. The same explanation applied to the males is as follows: the wild gray male has only one dose of black (since black is sex-linked). It has two doses of yellow. The hybrid has also one dose of black (B) but only one dose of yellow. It differs, therefore, from the wild male in having one dose of yellow instead of two. The darker color of the hybrid male would, in consequence, be due to relatively less yellow than that present in the wild male. The explanation is the same therefore for both sexes, but it involves the assumption that the color of the wild female which is the same as that of the male is due to the presence of a double dose of B and Y (BY, BY) while the color of the wild male is due to one dose of black and two of yellow (BY, Y). Removal of one Y from the wild female makes her darker; and similarly the removal of one Y from the wild male makes him darker also.

The numerical results for the F_2 generation are as follows:

$G \text{ } \varnothing \text{ by } B \text{ } \sigma^7 =$	$\begin{cases} G \text{ } \varnothing \\ G \text{ } \sigma^7 \end{cases} =$	$\begin{cases} \{ \\ \{ \\ \{ \\ \{ \end{cases}$	Gray \varnothing	5053
			Gray σ^7	4861
			Black \varnothing	1280
			Black σ^7	1385

The expectation, as shown by the analysis below, calls for three grays to one black. There are 9914 grays and 2665 blacks. The blacks fall considerably below expectation, yet the black flies are a vigorous strain and appear in the cultures to breed as well as the grays.

It will be noted that while the gray males run some 200 flies behind the females, the black males exceed the females by 100 flies.

Since these counts are from more complex crosses involving white eyes and short wings as well as red eyes and long wings,

and since the former characters are associated at times with diminishing returns, I give in the next table some results where only black and gray color are involved:

$$G \text{ } \varphi \text{ by } B \text{ } \sigma = \begin{cases} G \text{ } \varphi \\ G \text{ } \sigma \end{cases} = \begin{cases} \text{Gray } \varphi & \dots\dots\dots 867 \\ \text{Gray } \sigma & \dots\dots\dots 811 \\ \text{Black } \varphi & \dots\dots\dots 201 \\ \text{Black } \sigma & \dots\dots\dots 180 \end{cases}$$

There are 1678 grays and 381 blacks. The ratio is approximately four to one which is not very different from the preceding ratio. The analysis of this cross is as follows:

	Gray φ BYBrX — BYBrX
	Black σ ByBrX — byBr
F_1	Gray φ BYBrX — ByBrX
	Gray σ BYBrX — byBr
Gametes of F_1	BYBrX — ByBr
	BYBrX — ByBrX — byBr — byBr
F_2	Gray φ 3 Black φ 1
	Gray σ 3 Black σ 1

The reciprocal cross, gray male by black female gave the following results:

$$B \text{ } \varphi \text{ by } G \text{ } \sigma = \begin{cases} \text{Gray } \varphi \\ \text{Gray } \sigma \\ \text{Black } \varphi \\ \text{Black } \sigma \end{cases} = \begin{cases} \text{Gray } \varphi & \dots\dots\dots 1465 \\ \text{Gray } \sigma & \dots\dots\dots 1453 \\ \text{Black } \varphi & \dots\dots\dots 344 \\ \text{Black } \sigma & \dots\dots\dots 299 \end{cases}$$

The expectation is here also three grays to one black. The total for the grays is 2918, and for the blacks 643, which is about $4\frac{1}{2}$ to 1. In the grays, the males and females are nearly equal, while in the blacks, the females exceed the males by a fair margin. Since these numbers also are derived from mixed counts (as before) I give below a count involving only the two colors in question:

$$B \text{ } \varphi \text{ by } G \text{ } \sigma = \begin{cases} \text{Gray } \varphi \\ \text{Gray } \sigma \end{cases} = \begin{cases} \text{Gray } \varphi & \dots\dots\dots 836 \\ \text{Gray } \sigma & \dots\dots\dots 875 \\ \text{Black } \varphi & \dots\dots\dots 209 \\ \text{Black } \sigma & \dots\dots\dots 148 \end{cases}$$

There are 1711 grays and 357 blacks which is very nearly 5 to 1.

Wild (gray) by yellow

The results of this cross have been already published (1911), but may be given here for the sake of completeness. In both cases the parents were alike except for body color:

$$G \text{ } \varnothing \text{ by } Y \text{ } \sigma^8 = \begin{cases} \text{Gray } \varnothing & 654 \\ \text{Gray } \sigma^8 & 705 \end{cases} = \begin{cases} \text{Gray } \varnothing & \dots\dots\dots 525 \\ \text{Gray } \sigma^8 & \dots\dots\dots 340 \\ \text{Yellow } \sigma^8 & \dots\dots\dots 194 \end{cases}$$

The sum of the males is here nearly equal to the females as called for in the expectation (see below). It will be noted, however, that the gray males in F_2 greatly exceed the yellow males, and that the gray males are considerably more than half (268) the gray females.

It may seem that the discrepancy in the yellow males is not due to their viability alone, but rather that the gray-bearing male-producing spermatozoa are more likely to fertilize the eggs than are the yellow bearing sperm. The analysis is as follows:

	Gray \varnothing BYBrX — BYBrX
	Yellow σ^8 bYBrX — bYBr
<hr/>	
F_1	Gray \varnothing BYBrX — bYBrX
	Gray σ^8 BYBrX — bYBr
<hr/>	
F_2	Gray \varnothing 2
	Gray σ^8 1
	Yellow σ^8 1

The reciprocal cross is as follows:

$$Y \text{ } \varnothing \text{ by } G \text{ } \sigma^8 = \begin{cases} G \text{ } \varnothing & 397 \\ G \text{ } \sigma^8 & 282 \end{cases} = \begin{cases} \text{Gray } \varnothing & \dots\dots\dots 346 \\ \text{Gray } \sigma^8 & \dots\dots\dots 259 \\ \text{Yellow } \varnothing & \dots\dots\dots 226 \\ \text{Yellow } \sigma^8 & \dots\dots\dots 230 \end{cases}$$

The excess of females in F_1 is noticeable. The expectation in F_2 is equal numbers for all classes. The grays run ahead of the yellows. There is a very noticeable deficiency in the gray

males compared with the gray females. The analysis is as follows:

	Yellow ♀	bYBrX — bYBr	♀
	Gray ♂	BYBrX — bYBr	♂
	Gray ♀	bYBrX — BYBrX	♀
F ₁	Gray ♂	bYBrX — bYBr	♂
F ₂	Yellow ♀	1	
	Yellow ♂	1	
	Gray ♀	1	
	Gray ♂	1	

Black by yellow

Black females mated to yellow males give gray females and males. The data for F₂ are:

B ♀ by Y ♂ =	{ G ♀ = G ♂ =	Gray ♀	5147
		Gray ♂	2451
		Black ♀	1591
		Black ♂	750
		Yellow ♂	1957
		Brown ♂	530

The expectation is for the females, gray 6, black 2; and for the males, gray 3, black 1, yellow 3, brown 1. There are nearly 374 more gray females than 3 times the black females. There are also 500 more gray males than yellow males. Both excessive classes correspond to the F₁ classes. There are more black females than two times the black males. The analysis is as follows:

	Black ♀	ByBrX — ByBrX	♀
	Yellow ♂	bYBrX — bYBr	♂
F ₁	Gray ♀	ByBrX — bYBrX	♀
	Gray ♂	ByBrX — bYBr	♂
Gametes of F ₁	BYBrX — ByBrX — bYBrX — byBrX	♀	
	BYBrX — ByBrX — bYBr — byBr	♂	
	Gray ♀	6	Gray ♂ 3
	Black ♀	2	Black ♂ 1
			Yellow ♂ 3
			Brown ♂ 1

The reciprocal cross yellow females by black males gives gray females and yellow males. The numerical data follow:

Y ♀ by B ♂ =	{ G ♀ Y ♂ =	{ Gray ♀	2442
		{ Gray ♂	1893
		{ Black ♀	1893
		{ Black ♂	723
		{ Yellow ♀	1547
		{ Yellow ♂	1548
		{ Brown ♀	441
		{ Brown ♂	428

The expectation for the females and males alike is gray 3, yellow 3, black 1, brown 1. There are about 900 more gray females than yellow females; while the gray males are only 350 more numerous than the yellow males. The gray females and yellow males are the F_1 classes. The yellow females are as numerous as the yellow males. The black males are about a third of the gray males and more than this ratio in regard to the yellow males. The blacks run well ahead of the browns, the females being more than four times as numerous. The analysis is as follows:

	Yellow ♀	bYBrX — byBrX	
	Black ♂	ByBrX — byBr	
F_1	Gray ♀	bYBrX — ByBrX	
	Yellow ♂	bYBrX — byBr	
Gametes of F_1	byBrX — bYBrX —	ByBrX — BYBrX ♀	
	byBrX — bYBrX —	byBr — bYBr ♂	
F_2	Brown ♀ 1	Brown ♂ 1	
	Yellow ♀ 3	Yellow ♂ 3	
	Black ♀ 1	Black ♂ 1	
	Gray ♀ 3	Gray ♂ 3	

Wild (gray) by brown

When the normal (Gray) females were mated with brown males all the offspring were gray. The numerical* results for F_1 and F_2 were as follows:

G ♀ by Br ♂ = $\begin{cases} \text{G ♀ 266} \\ \text{G ♂ 236} \end{cases}$ =	Gray ♀	2618
	Gray ♂	1342
	Black ♀	765
	Black ♂	351
	Yellow ♂	985
	Brown ♂	300

The expectation is six gray females to two black females, and for the males 3 gray, 3 yellow, one black, one brown. The normal females are almost exactly twice the number of normal males. The normal males exceed three times the black males by nearly 300; and the black males exceed the brown males 50 flies. The yellow males are somewhat more than three times as numerous as the brown males, but less than three times as numerous as the black males. The yellow males, which should be as numerous as the normal males are about 350 fewer. The sum total of all the females is 3383 and of the males 2988. The males are about 400 flies fewer than the females.

Despite these differences the numbers accord fairly well with the expectation, at least the classes stand in the same general relation that the analysis calls for. The analysis follows:

	Gray ♀	YBBrX — YBBrX
	Brown ♂	yBBrX — ybBr
<hr/>		
F_1	Gray ♀	YBBrX — ybBrX
	Gray ♂	YBBrX — ybBrX
<hr/>		
Gametes of F_1	yBBrX — YBBrX — ybBrX — YbBrX	♀
	yBBrX — YBBrX — ybBr — YbBr	♂
<hr/>		
F_2	Black ♀	2
	Black ♂	1
	Gray ♀	6
	Gray ♂	3
	Yellow ♂	3
	Brown ♂	1

The reciprocal cross, brown females by gray males gave gray females and yellow males. The numerical data for the F_1 and

F₂ generation are as follows. The counts of the F₁ flies are taken from another similar cross.

Br ♀ by G ♂ =	{ G ♀ 124 Y ♂ 111 =	{	Gray ♀	406
			Gray ♂	171
			Black ♀	74
			Black ♂	55
			Yellow ♀	162
			Yellow ♂	190
			Brown ♀	37
			Brown ♂	51

Owing to the failure of many of the brown females to breed the numerical results are small. The expectation for the females is gray 3, black 1, yellow 3, brown 1, and for the males, gray 3, black 1, yellow 3, brown 1. It will be observed that the gray females greatly exceed the yellow females while the yellow males exceed slightly the gray males. The F₁ females are gray and the males yellow.¹ The analysis follows:

	Brown ♀	y ^b BrX - y ^b BrX
	Gray ♂	YBBrX - YbBr
<hr/>		
F ₁	Gray ♀	y ^b BrX — YBBrX
	Yellow ♂	y ^b BrX — YbBr
<hr/>		
Gametes of F ₁	YbBrX — y ^b BrX — YBBrX — y ^b BrX ♀	
	YbBrX — y ^b BrX — YbBr — y ^b Br ♂	
<hr/>		
F ₂	Gray ♀ 3	Gray ♂ 3
	Black ♀ 1	Black ♂ 1
	Yellow ♀ 3	Yellow ♂ 3
	Brown ♀ 1	Brown ♂ 1

Black by brown

When black females are mated to brown males all the offspring are black. The numerical data for F₁ and F₂ are as follows:

B ♀ by Br ♂ =	{ B ♀ 299 B ♂ 274 =	{	Black ♀	6124
			Black ♂	3015
			Brown ♂	2472

¹ In another experiment the gray males equalled the gray females.

The expectation is that the black females shall be as numerous as the sum of the two classes of males; there are about 600 too few males owing largely to a deficit in the brown class which runs about 450 behind the black males. The analysis follows:

	Black ♀ yBBrX — yBBrX
	Brown ♂ ybBrX — ybBr
F ₁ and gametes of F ₁	Black ♂ yBBrX — ybBrX Black ♀ yBBrX — ybBr
F ₂	Black ♀ 2 Black ♂ 1 Brown ♂ 1

The reciprocal cross, brown females and black males gives black females and brown males. The numerical data are as follows:

$$\text{Br ♀ by B ♂} = \begin{cases} \text{B ♀} \\ \text{Br ♂} \end{cases} = \begin{cases} \text{Black ♀} \dots\dots\dots 2191 \\ \text{Black ♂} \dots\dots\dots 1987 \\ \text{Brown ♀} \dots\dots\dots 1532 \\ \text{Brown ♂} \dots\dots\dots 1448 \end{cases}$$

The expectation is equality throughout; but the Browns run, class for class, about 600 behind the Blacks. The analysis gives:

	Brown ♀ ybBrX — ybBrX ♀
	Black ♂ yBBrX — ybBr ♂
F ₁	Black ♀ ybBrX — yBBrX ♀ Brown ♂ ybBrX — ybBr ♂
F ₂	Brown ♀ 1 Brown ♂ 1 Black ♀ 1 Black ♂ 1

Yellow by brown

Yellow females by brown males give yellow females and males. The numerical data are:

$$Y \text{ } \varnothing \text{ by Br } \sigma^7 = \begin{cases} Y \text{ } \varnothing & 115 \\ Y \text{ } \sigma^7 & 99 \end{cases} = \begin{cases} \text{Yellow } \varnothing & \dots\dots\dots 2295 \\ \text{Yellow } \sigma^7 & \dots\dots\dots 2232 \\ \text{Brown } \varnothing & \dots\dots\dots 830 \\ \text{Brown } \sigma^7 & \dots\dots\dots 758 \end{cases}$$

The expectation is three yellows to one brown, and the numbers approximate to this relation. When the difficulties of separating these two classes is taken into account the agreement is remarkably close. The analysis follows:

	Yellow \varnothing YbBrX — YbBrX
	Brown σ^7 ybBrX — ybBr

	Yellow \varnothing YbBrX — ybBrX \varnothing
F ₁	Yellow σ^7 YbBrX — ybBrX ybBr — YbBr

	Yellow \varnothing 3
	Yellow σ^7 3
F ₂	Brown \varnothing 1
	Brown σ^7 1

The reciprocal cross, brown females by yellow males, gives yellow females and males. The numerical data are:

$$Br \text{ } \varnothing \text{ by Y } \sigma^7 = \begin{cases} Y \text{ } \varnothing & 129 \\ Y \text{ } \sigma^7 & 102 \end{cases} = \begin{cases} \text{Yellow } \varnothing & \dots\dots\dots 1181 \\ \text{Yellow } \sigma^7 & \dots\dots\dots 1409 \\ \text{Brown } \varnothing & \dots\dots\dots 571 \\ \text{Brown } \sigma^7 & \dots\dots\dots 520 \end{cases}$$

The expectation is again three yellows to one brown; and this is fairly well realized. There are more yellow males than yellow females, and slightly more brown females than brown males. The analysis is as follows:

	Brown \varnothing ybBrX — ybBrX
	Yellow σ^7 YbBrX — YbBr

	Yellow \varnothing ybBrX — YbBrX
F ₁	Yellow σ^7 ybBrX — YbBrX — YbBr — ybBr

	Yellow \varnothing 3
	Yellow σ^7 3
F ₂	Brown \varnothing 1
	Brown σ^7 1

DISCUSSION

The color of the body of the wild fly appears from the experimental data to be due to at least three factors viz., yellow, black, brown. It has been shown in a former paper that the red eye of the wild fly is also due to the presence of three factors viz., vermillion, pink, orange. In both series one at least of the three factors is sex-linked; the factor for black in the one and for pink in the other. In crossing both series give almost parallel results. In the eye-color series the factor for orange is always present, either simplex or duplex. In my former paper I could not determine whether it is sex-linked or not, because it had never dropped out, but since then I have obtained a new mutation in which orange has dropped out, and, by suitable experiments, it has been shown that this factor also is sex-linked. It appears then that in the eye color series there are two sex-linked factors, *P* and *O*, and one not sex-linked, *V*. In the present series brown occupies a similar position in the symbolism used to that of the orange factor in the eye color series, but on the basis of this similarity it would not be justifiable to conclude that the brown factor is sex-linked.

In this connection I may record that during the summer of 1910 there appeared for a time, in one of my cultures, flies that had almost no color in the body although the eyes were red. A few pigment granules brownish in color were scattered over the abdomen. The flies resembled in some respects flies that had just emerged from the pupa case. The flies were extremely weak and died after a few days without progeny. Whether they represent the loss of the brown factor, or of the color producer can not be stated. Since they appeared in cultures of gray flies the latter interpretation seems more probable.

Whether the comparison drawn above between the eye color series and the body color series has any real significance can, of course, be only a matter of conjecture. It should be pointed out that any eye color may be combined with any body color, and I have been unable to detect any correlated effect of these two combinations upon each other, except such effect as is due to color contrast.

One can not work with these body colors without being impressed by the similarities between the brown and the black flies on the one hand, and the yellow and the gray on the other. Brown and black lack the yellow factor, and if this, as I suppose, acts to some extent as an inhibitor the resemblance is manifest; while conversely the presence of the yellow factor in the yellow and in the normal fly makes clear their resemblance. One is tempted to surmise that black and brown may both be stages of the same chemical reaction, which surmise would be more probable if it could be shown that both factors are contained in the X chromosome, but this relation in itself could not be used as an argument to urge their dependent chemical nature.

PLATE 1

EXPLANATION OF FIGURES

- 1 Normal or gray female (the outer marginal vein is slightly exaggerated in the figure).
- 2 A black female.
- 3 A brown female.
- 4 A yellow female. The contrast between the black, yellow, and brown flies is well brought out in the figures.



EXPERIMENTS ON THE REPRODUCTION OF THE HYPOTRICHOUS INFUSORIA

I. CONJUGATION BETWEEN CLOSELY RELATED INDIVIDUALS OF STYLONYCHIA PUSTULATA

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TWENTY FIGURES (ONE PLATE)

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I. INTRODUCTION

Maupas ('88, '89), from his investigations on the phenomenon of conjugation in *Stylonychia pustulata*, believed that conjugation, in order to be fertile, must take place when the organisms were at a certain stage of maturity which, in this species, occurred between the 130th and 170th generations. Conjugation could occur between gametes after this so-called period of sexual maturity, but in such cases the union was infertile, and the ex-conjugants would invariably die soon after separation. Provided conjugation did

not occur before the 170th generation senile degeneration set in, resulting in an abnormal diminution in size of the organisms and other pathological conditions and culminating in a complete disappearance of the micronuclei. This senile degeneration reached its climax at the 316th generation at which point all the non-conjugants would die. This author also held that conjugation in order to be fertile must occur between gametes of diverse ancestry, and that the phenomenon would not take place except when the animals were in a medium in which there was a scarcity of food.

Maupas believed that these requirements were necessary for fertile syzygies not only in *S. pustulata* but also, with some differences in time of sexual maturity, for all Infusoria and that senile degeneration would always occur provided conjugation did not take place during the so-called period of maturity. He upheld then the view that rejuvenation by means of conjugation was necessary for the continued existence of the organisms. In case it did not occur the life cycle would end after a fairly definite number of generations.

Joukowsky ('98) found, as had been previously announced by Bütschli ('76), that in *Paramacium putrinum* fertile syzygies could occur between individuals only a very few generations removed from conjugation. In a number of instances only seven or eight generations elapsed before fertile unions occurred between descendants of a single isolated ex-conjugant. This investigator also kept *Pleurotricha* through 458 generations in eight months without the occurrence of either conjugation or degeneration.

Calkins ('02a) in his study of the life history of *Paramacium caudatum* found that fertile syzygies occurred at the 350th, 410th, 467th and 500th generations, thus showing that, in this form at least, the so called period of sexual maturity really has no significance. He also showed that conjugation between closely related gametes was as generally fertile as in the cases where the gametes were of diverse ancestry. Scarcity of food was shown to be not a sufficient factor to induce conjugation and finally, while some evidences of degeneration were noted in the animals of the cultures at times, such as low division rate, diminished size, and occurrence

of monsters, the micronuclei were always present. The investigations on this form, however, gave evidence that the life cycle was essentially a limited one but that various chemical stimuli could be substituted for conjugation to 'rejuvenate' the animals and in this way the life history could be prolonged to a great degree. Eventually, however, the organisms reached a stage where it was found impossible to stimulate them further and the cultures died out.

Work by Woodruff ('05) on a number of the hypotrichous infusoria, Popoff ('07) on the *Stylonychia mytilus*, and Gregory ('09) on *Tillina magna* all gave additional support to the view that the life cycle of these forms is a limited one. Both Woodruff and Gregory were able to prolong the life of the cultures somewhat by artificial stimulation.

Enriques ('05) from his investigations on a number of species of the Infusoria, including *S. pustulata*, came to the conclusion that the degeneration which he observed in some cases was not a so-called senile degeneration due to 'protoplasmic' old age but a degeneration caused by the toxic influences of certain bacterial poisons in the culture media.

Recent investigations by Woodruff ('08, '12) with *Paramaecium aurelia* which he has kept on a 'varied environment' medium¹ have shown that this organism can be bred indefinitely without conjugation or artificial stimulation, thus furnishing conclusive evidence of the unlimited power of reproduction of an infusorian without the need of 'rejuvenescence' when suitable culture conditions are supplied.

Jennings ('10), also working with *Paramaecium*, found no evidence to support Maupas' view of the infertility of conjugation occurring between closely related gametes.

¹ Woodruff ('08) thus described the 'varied environment' medium: "It was found that *Paramaecium* can exist in nearly any infusion which may be made from materials collected in ponds and swamps, and accordingly, in the hope of supplying as far as possible all the elements which may be encountered in the usual habitat of the organism, water was taken from ponds, laboratory aquaria, etc., together with its animal and plant life. In other words, no definite method was employed in selecting the material, but it was simply collected at random from many sources, thoroughly boiled, and then used."

To summarize briefly, then it has been shown by a number of investigators working on several species of Infusoria that Maupas was incorrect in asserting (1) that the Infusoria have a definite life cycle in which can be distinguished a period of sexual maturity during which time conjugation will be fertile and if conjugation does not take place, a resulting period of 'senile degeneration' finally terminating with the death of the organism after a certain number of generations, (2) that conjugation, in order to be fertile, must be between gametes of diverse ancestry. Finally, the work of Woodruff with *Paramecium aurelia* has shown that it is possible to breed this organism indefinitely without conjugation or artificial stimulation.

Inasmuch as *Stylonychia pustulata* has been shown to be a species well adapted to the treatment demanded in laboratory cultures and also because some of Maupas' conclusions which have been shown not to hold for other of the Infusoria have not been re-investigated in the species which he used, it was decided to make use of this species in these experiments. In the present paper is presented a study of the following points:

1. The life history of *Stylonychia pustulata* when bred on a 'constant' culture medium of beef extract and a culture medium of hay infusion.
2. The morphological and physiological changes during the life of the organism when bred on these culture media.
3. The relation of conjugation to the environment of the organisms.
4. The effect of conjugation between closely related individuals which have had an identical environmental history.

The writer is glad of the opportunity to express his great indebtedness to Professor Lorand L. Woodruff not only for suggesting the problem but also for his advice and helpful criticism during the entire course of the work.

II. METHODS

In order to study the life history of a number of the Protozoa, Maupas isolated an individual in a favorable culture medium and in the course of a few days, when a large number of animals had arisen by fission, he counted the individuals, computed and recorded the number of divisions that had taken place, and isolated one of the animals in another dish with fresh culture medium to continue the culture. In some cases over 900 individuals were allowed to accumulate before the isolation took place. Bütschli later pointed out that there were two sources of error present in such a method, namely, the difficulty of securing an accurate count of so many organisms on a preparation at one time and the assumption that the rate of division had been the same for all the animals in the cultures, which might or might not be true depending entirely upon the physiological condition of the different individuals of the culture.

Calkins ('02) in his work with *Paramecium caudatum* modified Maupas method in order to overcome these difficulties. Instead of using a large receptacle and allowing the animals to accumulate in large numbers before isolation, he kept them on glass slides having a central depression holding about five drops of the culture medium, and from these slides he isolated an individual every one or two days so that only a few were present at the time of isolation. This method of isolation, besides giving the exact number of generations through which the culture has passed, also prevents conjugation occurring in the slides of the main lines which are isolated daily. This method very little modified has since been used by a number of investigators, and notably by Woodruff in his work with *Paramecium aurelia*. The cultures discussed in this paper have all been conducted by this same method and as it has now become well-known only a brief description of the process will be given.

The animals to be studied were isolated on glass slides having a central depression large enough to hold five drops of water. The slides to prevent evaporation were kept in moist chambers made from large Stender dishes with ground glass covers, and each having a capacity of eight slides. In starting a culture, an animal was

isolated on a depression slide in some of the culture medium and placed in the moist chamber. When it had produced four individuals by division, these were isolated and thus the four main lines of a culture were started. These four lines were examined daily, the number of divisions observed and recorded and one animal isolated from each to continue the main lines. The slides from which the isolation took place were saved as stock in order to replenish the main lines in case any of the individuals isolated were lost through accident. In this work three days stock was kept which, with the main lines, made in all sixteen slides under observation in a culture. For isolation, capillary pipets were made use of, a separate one being reserved for each culture. In all the isolation work the greatest care was taken to prevent contamination of the cultures. All pipets and slides that were used were carefully sterilized immediately before using. A dissecting microscope with a 10 multiple lens was used in all the isolation work. Permanent preparations of the individuals from the cultures were made from time to time. The method used was that of Calkins ('02a) and Woodruff ('05).²

Two culture media were employed; a 'constant' medium which consisted of a 0.025 per cent. solution of Liebig's extract of meat, and a hay infusion medium. There are at least two essential conditions for a 'constant' culture medium. It must contain the elements necessary for the maintenance of protoplasm and it must be a medium in which bacteria will develop readily, in order to provide food for the animals. A solution of beef extract fulfils these requirements and previous work by other investigators having shown that strong solutions of beef extract will artificially stimu-

² This method is given by Woodruff as follows: "The specimen to be preserved is isolated by means of a fine-pointed pipet on a clean depression slide (which is kept just for this purpose) with as little of the culture medium as possible. To this is added three or four drops of bichlorid of mercury in saturated solution with 5 per cent. of glacial acetic acid. After about five minutes the specimen is transferred to another slide and a few drops of 75 per cent alcohol is added. A slide is now smeared with a trace of egg albumin and the specimen is taken from the 75 per cent alcohol and gently spurted on to the albumin. After a short time, when the alcohol has coagulated the albumin, the slide with the specimen adhering to it is transferred to a jar of 75 per cent. alcohol and thereafter treated by the ordinary slide method."

late various species of Infusoria during periods of depression, it appeared probable that if a solution of the proper strength was used that a beef extract solution would provide a 'constant' medium in which the organisms would thrive. In order to determine the proper strength of solution to be used a series of preliminary experiments was undertaken. As a result it was found that a solution of 0.025 per cent gave the best results. Accordingly a large quantity of the solution was made at the beginning of the work, and placed in test tubes (25-50cc. in each), and these were then plugged with cotton and sterilized. The solutions in the test tubes remained sterile until placed on the slides.³ The hay infusion was made by placing about one gram of hay in 85cc. of tap water and then raising the temperature of the mixture to the boiling point. The infusion thus made was used for three or four days afterwards. No attempt was made to use the same kind of hay at all times or to have the infusion of exactly the same strength.

The graphs in this paper showing the rate of division were computed from the sum of all the divisions in the four lines of a culture for the period stated, e.g., a total of 80 divisions in ten days for the four lines of a culture is 20 divisions in ten days for one line, or exactly two divisions per day. In such a graph we get the average rate of four lines again averaged for ten or thirty-day periods as the case may be. By such a method individual peculiarities and tendencies are largely eliminated and the curve of the graph shows the average division rate of the animals in the four lines of a culture.

III. MATERIAL

A specimen of *Stylonychia pustulata* was found in a laboratory hay infusion, September 21st, 1910. It was isolated on a regular depression slide and a few drops of hay infusion added. Within the next twenty-four hours two divisions occurred producing four individuals. Each of these was isolated on a separate slide and the four lines of the culture were thus started. After the culture

³ For further information in regard to the use of beef extract as a culture medium and the effect of its use in a culture of *Paramecium aurelia*, the reader is referred to the paper by Woodruff and Baitsell ('11).

had been running ten days, the medium was changed to a 0.025 per cent solution of Liebig's extract of meat and the culture was designated Sb. From this time (October 2, 1910) culture Sb was kept on the 'constant' medium of beef extract. As far as one

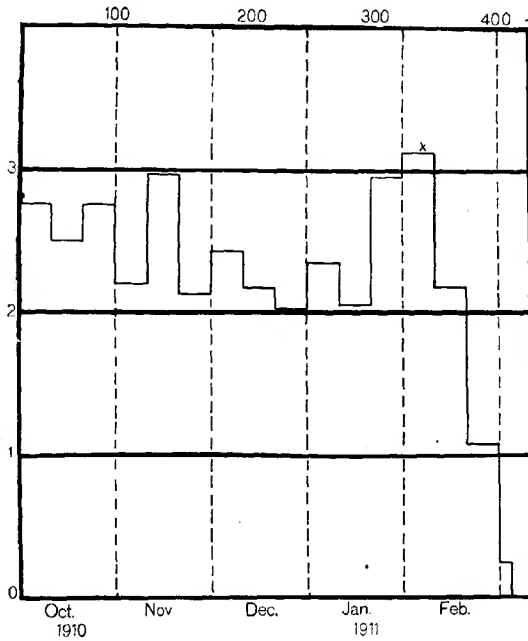


Diagram 1 Graph giving the life history of Culture Sb, showing the average daily rate of division of the four lines of the culture again averaged for ten day periods. Beef extract culture medium. Point marked X indicates the point at which conjugation appeared in the stock of the culture.

could judge by appearances of every kind, the medium was a favorable one for this animal. The division rate of the culture when averaged for ten day periods was never as low as two divisions per day during the first four months and at times the division rate exceeded three divisions per day when averaged for the same period

of time (diagram 1). At the 150th generation a sub-culture, designated Sb_h, was started from this original culture (diagram 4), and this sub-culture was kept in a hay infusion medium. Both of these cultures thrived until at the 350th generation conjugation occurred in the stock of the Sb culture (beef medium). Following the occurrence of conjugation in this culture the rate of division of the main lines rapidly decreased and the culture died out about three weeks later at the 403rd generation (diagram 1). Conjugation did not occur in the Sb_h culture (hay medium) and, following the death of the Sb culture, another sub-culture was started by isolation from the Sb_h culture (diagram 4) and this culture, designated Sb_{hb}, was placed on the beef medium. This last named culture was continued on the beef medium for about three and one-half months when conjugation occurred in the stock. As in the first beef culture (Sb) the main lines of the Sb_{hb} culture, coincident with the appearance of conjugation, began to decrease in vitality, as indicated by the fission rate, and about three weeks later all the animals of the culture had died (diagram 3). The Sb_h culture on the hay infusion continued without conjugation but finally died out at the 572nd generation (diagram 2).

IV. MORPHOLOGY AND PHYSIOLOGY OF THE NON-CONJUGANTS

A. Living material

In the February epidemic, conjugation made its appearance in the stock of the Sb culture at a time when the animals were dividing at the most rapid rate to which they had attained during the entire period of these experiments (diagram 1, point marked X). On account of this fact a slide of three or four days standing would as a rule have a number of animals varying from 100 to 200, and during the epidemic, conjugating animals could always be found on such slides. The phenomenon of conjugation did not occur in the main lines of the culture inasmuch as only a few animals were present on a slide of a day's standing. The point that should be emphasized is that the phenomenon was of very general occurrence on any slide on which were a sufficient number of animals.

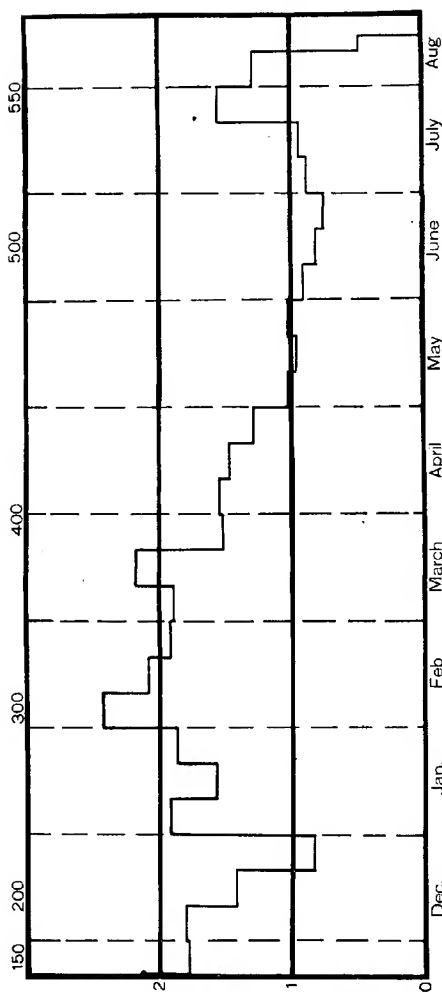


Diagram 2 Graph giving the life history of Culture Sbh, showing the average daily rate of division of the four lines of culture again averaged for ten day periods. Hay infusion culture medium.

After the epidemic had been present for a few days it became evident that the culture was not thriving as it had been previously. This was shown chiefly in two ways: (1) The fall in the division rate. At the time of the appearance of conjugation in the stock culture, the animals in the main lines were dividing at an average rate of over three divisions per day. For the ten days following the appearance of the phenomenon the average rate fell

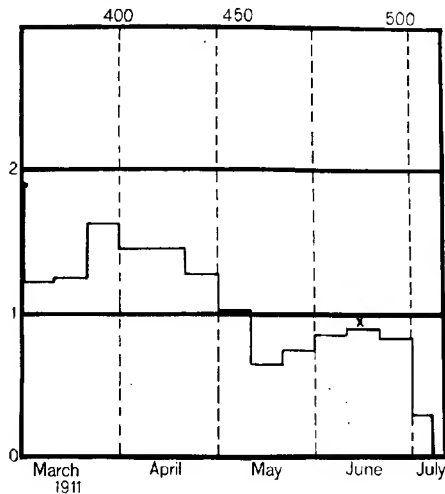


Diagram 3 Graph giving the life history of Culture Shbb, showing the average daily rate of division of the four lines of culture again averaged for ten day periods. Beef extract culture medium. Point marked X indicates the point at which conjugation appeared in the stock culture.

to approximately two divisions per day. In the next ten days the rate fell to an average of one division per day and this was followed by the death of the culture on the third day following (diagram 1). This fall in the division rate could be noted not only in the slides constituting the main lines of the culture but also in the stock slides. Soon after the appearance of conjugation the slides of stock in many instances instead of having a large number of individuals present would have only a very few. In some cases all

the animals on a stock slide would die in the course of two or three days. In the other stock slides, on which a sufficient number of animals were present, numerous pairs of conjugants would be seen. (2) The appearance of the animals in the culture. Conjugation had not been present in the culture many days before it could plainly be seen that in many of the non-conjugants a degenerative process was taking place. Such animals were more sluggish, not normal in shape and they were considerably darker in appearance. In the days just preceding the death of the culture this condition became more and more intensified and it became increasingly difficult to find normal, active animals in the culture. Many of the animals isolated in the main lines, instead of dividing, would degenerate and die. An apparently normal individual isolated in the morning would, in many instances, by the afternoon of the same day have passed through degenerative stages resulting in a decrease in size, change of shape, decrease in activity, etc. Examined again later in the evening of the same day it was very evident that these changes were more intensified and they generally resulted in the death of the animal that same night. This degeneration was observed in a very large number of non-conjugants especially in the days shortly before the death of the culture.

As soon as it was noted that the division rate of the culture was rapidly falling, various other media were used in an endeavor to stimulate some of the animals. The Sb culture was continued on the beef-extract medium as formerly but from it sub-cultures were started by isolation and placed on a number of other media such as hay infusions of varying strengths, the 'varied environment' medium, and solutions of beef extract of different strengths. The effect of different temperatures was also tried. From all these experiments the results obtained were entirely negative, none of the sub-cultures thriving as well as the original culture kept on the regular beef medium and all dying out within a few days. In a number of instances the change of medium caused an almost instantaneous death. The protoplasm of the non-conjugants appeared to be in a condition in which it was impossible for it to withstand any changes. The fact that the Protozoa, when in a weakened condition, are unable to endure a change of medium has

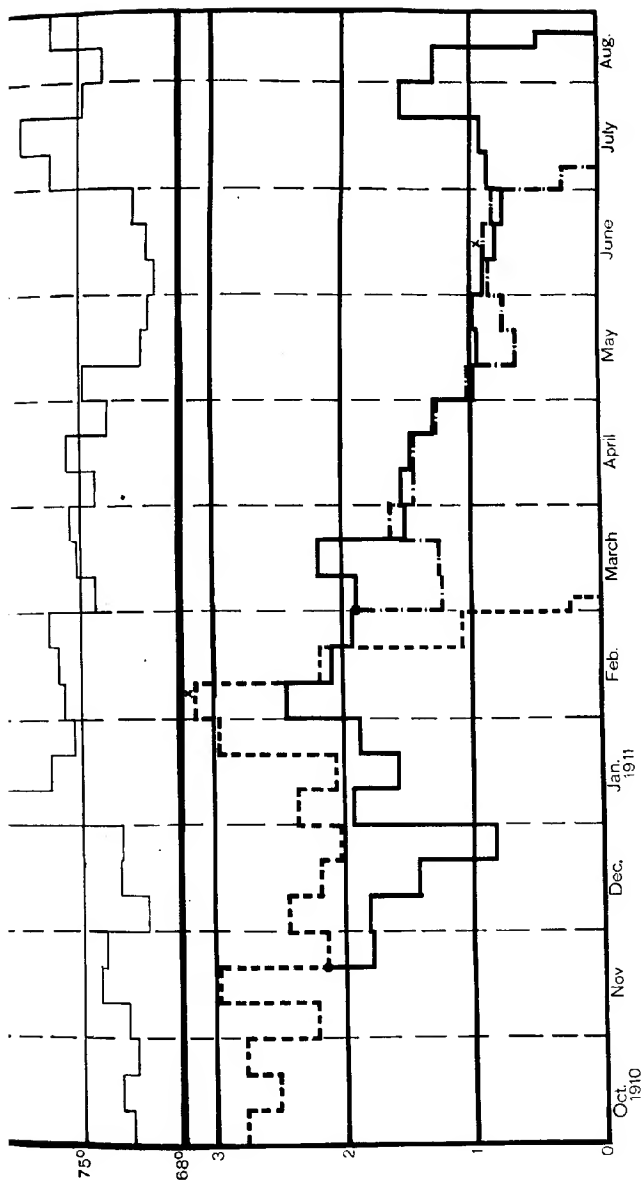


Diagram 4 Graph giving the entire life history of the three cultures of *S. pustulata*, viz., Sb, SbH, and SbHb. It shows the daily average rate of division again averaged for ten day periods. The upper graph gives the average room temperature (°F.) for the corresponding periods. Points marked X indicate the points at which conjugation appeared in the stock of the Sb and SbHb cultures.

been noted previously by other investigators. Other experiments would have been tried in an endeavor to restore the non-conjugating animals to a normal condition had it not been for the fact that the number of animals in the culture became so reduced that none were available for additional experimental work.

The June epidemic of conjugation occurred in the stock of the second culture kept on the beef medium (Sbhb) from June 14, to July 5, 1911 (diagram 3 point marked X). This epidemic differed from the previous one in that the number of animals on any one slide was very much less than in the previous case, due to the less rapid division rate of this culture. In comparison with the Sb culture this one never attained a high rate of division and, at the time when conjugation appeared in the stock, the main lines were averaging a little less than one division per day. However, during the month previous to the appearance of conjugation there had been a gradual increase in the division rate from about one-half division to nearly one division per day. Because the animals divided so slowly a stock slide of a few days standing would rarely contain more than fifty animals and, in general, the number was considerably less than that. Nevertheless an epidemic of conjugation occurred and numerous pairs were observed on some slides which had as few as twenty-five animals present. As has been noted in the former epidemic, all the animals of the culture appeared to be in the same physiological condition and some slides were seen on which practically every animal was united in conjugation. Also there began in this culture, coincident with the appearance of conjugation in the stock, a marked and rapid decline in the fission rate of the main lines of the cultures and this decline ended in the death of the culture twenty days later. The appearance of the animals, the degenerative processes through which they passed and the entirely negative results obtained from endeavors to stimulate them by artificial means, all gave proof that, as in the first epidemic, some fundamental change had taken place in the animals of the culture. This change was of such a character as to produce conjugation whenever a sufficient number of animals were present on a slide. If the phenomenon was prevented the non-conjugants degenerated and died.

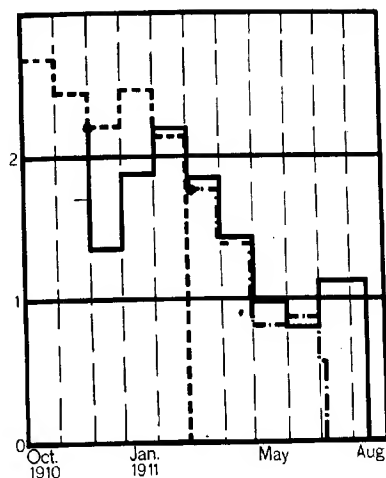


Diagram 5 Graph giving the entire life history of the three cultures of *S. pululata*, viz., Sb, Sbh, and Shhb. It shows the average daily rate of division again averaged for thirty-day periods.

B. Prepared material

A study of the permanent stained preparations of the non-conjugants of the Sb and the Shhb cultures gives conclusive evidence of the degeneration that occurred in these animals. There is a decrease in the size of the animals and a great change in the cytoplasmic and nuclear structure. In all the animals the micronuclei are present but in many instances they are found to be considerably enlarged and situated far from their normal positions. In most cases the macronuclei have undergone a marked change and have lost their characteristic shape. Figure 1 shows a normal individual taken from the Shhb culture. This specimen is quite typical of the animals to be found in the beef cultures before degeneration had set in. In figures 2 to 6 are to be seen non-conjugating individuals which were preserved at various stages of degeneration.

Abnormal animals had never occurred in any of the cultures kept on the beef medium previous to conjugation and in the culture kept on the hay infusion medium, in which no conjugation occurred during its entire history, entire freedom from abnormal and degenerate animals was noted. In figures 7 and 8 are shown two typical specimens from the hay culture. Figure 7 represents a characteristic specimen preserved at the time of the first epidemic of conjugation in the Sb culture, and figure 8 shows an animal preserved shortly before the death of the hay culture. In this last figure one of the micronuclei is seen in the stage of division. The other micronucleus is present and in the same stage, but is out of focus.

From the study of the living material it has been shown that the non-conjugating animals of the Sb and Sbhb cultures kept on the beef extract medium, coincident with the appearance of conjugation, began to degenerate and die. The prepared material gives proof that the degeneration resulted in marked morphological changes in both the nuclear and cytoplasmic structure.

V. MORPHOLOGY AND PHYSIOLOGY OF THE CONJUGANTS

A. *Living material*

In order to trace the effect of conjugation on the ex-conjugants, it was deemed necessary to isolate a large number of the conjugating animals. Accordingly, during the first epidemic, 132 pairs of conjugants were isolated, and in the second epidemic 20 more pairs were added, making in all 152 pairs which were isolated. More conjugants would have been isolated in the second epidemic but owing to the scarcity of the material it was impossible. Each pair of conjugants was drawn up with a fine pipet and then transferred to a sterile depression slide and about five drops of the beef extract medium added. Inasmuch as this was *exactly the same kind of medium* in which the animals had been kept previously and in which the conjugation had occurred, the *character of their environment was not changed* by the isolation. Consequently the possibility of any reaction of the conjugants due to the change of environment was *entirely eliminated*. Mention should also be made of the fact that, at the time of isolation, each pair of conjugants was examined

with a high magnification in order to determine if the conjugating animals were of normal appearance. The few pairs that did not appear normal were rejected and are not included in the totals given above. Of the 152 pairs of conjugants isolated, between 25 and 30 pairs were used in making permanent preparations so that approximately 125 pairs remained for observation. The work of isolation in both the epidemics of conjugation was carried on throughout the time that the phenomenon was occurring and the conjugating pairs were also isolated when the animals had been united for varying periods of time.

Careful study of the living conjugants entirely failed to reveal any evidence pointing to a pathological condition of the gametes at the time of conjugation. In neither shape nor size could any deviation from the normal be observed. It could be noted in some cases that the animals on a slide in which conjugation was occurring evinced remarkable activity. Calkins ('02a) found with *Paramecium caudatum* that at such periods the protoplasm of the animals became 'miscible' and that as many as eight or ten individuals would be found fused together in an irregular mass at times. Although it was very evident that conjugation would occur during these epidemics whenever opportunity was afforded, abnormal fusions of several animals were never observed.

Maupas ('89) gives the length of time during which the gametes of *S. pustulata* are together in conjugation as varying from twenty-two to thirty-nine hours according to the temperature; the shorter periods occurring with the higher temperatures. In this work the period was found to vary from twenty-four to thirty-six hours, thus corresponding very closely with the time given by Maupas. A very few cases were noted in which the animals had not separated at the end of thirty-six hours but it was clearly evident in such cases that the process was not normal and the resulting ex-conjugants never gave any promise of continued life. The records show that from over 90 per cent of the conjugating animals ex-conjugants were obtained which were at the time of separation, as far as could be judged from a careful and exhaustive study of the living material, normal in every respect and which gave every promise of continued life and multiplication. In brief, then, and

this fact should be emphasized, the phenomenon of conjugation which occurred in the two epidemics, judged by the study of the living material, gave every indication of being an entirely normal process.

In both the epidemics of conjugation, the syzygies were entirely between closely related gametes. An endeavor was made, by mixing animals from the stock slides of the conjugating cultures with animals from the hay culture, to secure conjugation between gametes which were not closely related and which had been under different environments. In both the epidemics this experiment was tried a number of times but without success. The animals from the hay culture were very evidently not in a state which would permit of conjugation occurring.

B. Prepared material

No attempt was made in this work to secure sufficient permanent stained preparations of the conjugating animals to permit working out the cytological details of conjugation in this species. The main object of the work was to study the physiological effects of the phenomenon and permanent preparations were made primarily to determine if a true process of conjugation had actually taken place. Maupas mentions that he found this form to be a very unfavorable one for the study of the cytology of conjugation and no doubt any investigator will agree with this statement. The nuclear changes which this form undergoes during the process of conjugation cannot be worked out with surety without securing an epidemic of conjugation in a mass culture and then making use of the imbedding and sectioning method as used by Calkins and Cull ('07) so successfully with *Paramaccium*.

However, all the prepared material which could be secured under the conditions of a pedigreed culture shows conclusively that a true process of conjugation actually took place. A study of the material reveals the fact that the *micronuclei* are present in every case and furthermore it appears that they are acting normally. The appearance of the conjugating animals with the nuclear structure is clearly shown in figures 9 to 12. In figure 9 is shown a

conjugating pair shortly after the union has occurred. In figure 10 the micronuclei have reached their maximum enlargement and are shown in the spindle stage of the first division. In the animal to the right, the micronuclei are in the same stage but are out of focus. In figure 11, in the animal to the right what is interpreted as the copulation nucleus can be seen in the spindle stage prior to the first division. The same stage is present in the other animal but it is out of focus. Figure 12 is in a stage just previous to the separation of the gametes.

The evidence presented both by the study of the living conjugants and the prepared material gives every indication that the conjugation which occurred in the beef cultures was a normal process and resulted in two ex-conjugants which, at the time of separation, gave every promise of continued existence.

VI. MORPHOLOGY AND PHYSIOLOGY OF THE EX CONJUGANTS

A. Living material

Resulting from the 125 pairs of conjugants isolated during the two epidemics, approximately 230 ex-conjugants were obtained which appeared to be normal at the time of separation. Shortly after separation they were quite as active as the regular non-conjugants of the cultures. They were a trifle smaller in size than the average individual but extensive study of the living material failed to show any other distinguishing characteristic.

When examined a few hours later, the ex-conjugants invariably showed that certain changes had taken place which may be summarized as follows: (a) A noticeable decrease in size had occurred. (b) The animals were considerably less active than formerly. (c) The characteristic avoiding reaction had almost entirely disappeared and their swimming movements consisted chiefly in turning on their short axis. (d) A change in the shape of the ex-conjugants had taken place and instead of having a normal elongated form, they were very nearly circular. (e) The animals had become quite dark in color.

If examined again, some eight or ten hours later, a considerable accentuation of these degeneration changes could be noted; again

a very noticeable decrease in size; a cessation of practically all locomotion had occurred and the animals were still more opaque. If examined with the low power, the ex-conjugants would appear more as motionless black specks than as living organisms. Within the next fifteen to eighteen hours the individuals would invariably die. The entire course of the degeneration occupied from thirty to thirty-six hours after the separation had occurred. Ex-conjugants which at the time of the separation in the morning appeared as active, normal individuals would, when examined in the afternoon of the same day, be found to be undergoing the degeneration as outlined above. By the following morning a large percentage of these would have disappeared and the remainder would be in a still further stage of degeneration. During the course of the day these would all die. *In all the large number of ex-conjugants under observation, over 230 individuals in all, not a single one lived forty-eight hours after separation and not a single one divided.*

The death of one of the ex-conjugants was observed under the microscope. This individual at the time of separation was apparently normal in every way. At the time of the observation, some thirty-three hours later, it was in the last stages of degeneration and under the high magnification appeared as a small, motionless dark object. The only evidence of life was a slight beating of a few cilia. The animal was in the regular beef medium and the observations were not such as to injure it in any way. After having been under observation for about one-half hour the animal suddenly appeared to explode and what had the second before been living protoplasm was now simply an amorphous mass.

In an endeavor to get some of the ex-conjugants to live a number of experiments were tried. Some were isolated, after separation, in various other media such as strong beef extract solution, hay infusions and the 'varied environment' medium. In all of these experiments only one of the ex-conjugants resulting from the separation of any one pair of conjugants was experimented with, the other ex-conjugant not being disturbed but simply left in the medium in which the separation had taken place. None of the methods used had any effect whatsoever in prolonging the lives of the ex-conjugants, all of them passing through similar degenerative

stages and no differences being noted between the ex-conjugants which were experimented with and their mates which remained in the same medium in which the separation had taken place. A great many of the slides were examined for cysts but none were found. That nothing injurious to the ex-conjugants was present upon the slides or in the medium in which they had died was shown several times by placing on the same slides and in the same medium in which the ex-conjugants had died, individuals from the stock of the culture. In every case they lived and divided.

From the fact, as noted above, that the ex-conjugants pass through certain degenerative stages and die within forty-eight hours after separation, conclusive evidence is furnished of the infertility of the conjugation which occurred in these cultures of *S. pustulata* which were kept on a beef medium.

B. Prepared material

Passing next to a study of the permanent stained preparations of the ex-conjugants, typical examples of which are shown in figures 13-15 it can be said that the micronuclei are present in all cases. Figure 13 shows a condition shortly after separation. Figure 14 represents an early stage in the degenerative processes, and figure 15 shows very clearly the appearance of an ex-conjugant during the last stage of this process.

It is evident, then, that the study of the prepared material of both the conjugants and the ex-conjugants shows that Maupas was incorrect in his statement that infertility of conjugation, in this form, is due to a loss of the micronuclei: and the results obtained in this investigation agree with those obtained by other investigators working on closely related species:

Split conjugation

Calkins (02a) in his work with *paramecia*, was able to separate the conjugants before the regular time and before there had been any interchange of nuclear material. This was done by drawing up the conjugating animals in a fine pipet and then ejecting them somewhat forcibly. From the 'ex-conjugants' thus obtained he

had a number of instances in which they lived and divided normally, thus showing conclusively that, even though the paramaecia were in the physiological state in which conjugation could occur, if they were separated before the supposedly essential factor of the process took place, i. e., the exchange of nuclear material, they could still live. Following the same method, split conjugation was brought about in these experiments. The operation was performed with animals that had been united only a few minutes, and which were fused for only a short distance at the anterior end. After the forcible separation, the resulting 'ex-conjugants' were examined carefully and were found to be normal in appearance. Three pairs of conjugants were separated and the resulting six individuals were isolated. This was done about noon. By evening of the same day it could be seen that they had changed very greatly and appeared to be in a stage closely resembling that described above as the first stage in the degeneration of the ex-conjugants. Three out of the six animals died that night and the remaining three during the next day. In these cases it appeared that the mere contact of the gametes without any interchange of nuclear material was sufficient to bring on a rapid degeneration and death.

VII. DISCUSSION AND CONCLUSIONS

Although the conjugation which occurred in both of the cultures kept on a beef medium in every instance resulted fatally, a careful study of both the living and prepared material has failed to show any reason why such should have been the case, but on the contrary such a study gives every evidence that the process which took place was normally effected.

The fact that conjugation is many times infertile and results in the death of the ex-conjugants when the gametes have been kept in laboratory cultures has been noted by a number of investigators. Calkins (02a), for example, shows that of 80 ex-conjugants of *Paramecium caudatum* from his laboratory cultures only five individuals, or 6 per cent were living at the end of thirty days; 37.5 per cent died without dividing and 60 per cent were dead within

ten days after separation. Entirely different results were obtained from three pairs of 'wild conjugants' of the same species. Of the six ex-conjugants obtained only one died and the remaining five thrived until the observations were discontinued. Cull ('07) also obtained similar results working with the same species. From 40 pairs of conjugants taken from laboratory cultures only 12 ex-conjugants or 15 per cent were alive at the end of the month; while from 93 pairs of 'wild conjugants' 70 per cent of the ex-conjugants were alive at the end of the same period. Both Bütschli ('76) and Calkins ('12), working with *Blepharisma*, have noted the infertility of conjugation. Bütschli did not succeed in getting any of the ex-conjugants to live longer than three days, and Calkins not longer than sixteen days, 74 per cent dying within five days after separation.

It has been suggested that the greater fertility of 'wild' conjugants than of those from a laboratory culture may be due either to a lack of vitality of the animals in the cultures, or to the fact that in conjugation, if it is to be fertile, there must needs be a new physico-chemical relation established by the fusion of the nuclei to furnish the factors necessary for the reorganization of the animals, and that the establishment of this relation is not possible in animals which have been subjected to identical conditions.

The first view cannot be held as an explanation of the fertility of conjugation in these experiments for in the February epidemic, conjugation made its appearance when the animals were dividing at the highest rate they had ever attained. Investigators are agreed that the rate of fission is an indication of the general vitality of the animals. Judged in this way, as well as by their general appearance and actions, they were in a perfect physiological condition at the time of the appearance of the first epidemic of conjugation. In the June epidemic inasmuch as the division rate had been considerably lower, it is probable that the general vitality of the animals was not as high as had been the case previously, but, at the time when either of the epidemics appeared, it certainly could not be said that the individuals of the cultures gave evidence of a low degree of vitality. The evidence presented from both

the cultures is such as to show that an *apparently* perfect physiological condition of the gametes at the time of conjugation is no guarantee that the syzygy will be a fertile one.

The other view, that the infertility of conjugation in animals which have been under identically the same conditions of environment is due to an inability to establish certain new relations, is a suggestive one and harmonizes with the idea first stated by Treviranus and later contended for by Weismann ('91), that the real purpose of conjugation is to bring about variations in the progeny. The work of Jennings ('11) with paramaecia in which he shows that the progeny of the conjugants are more variable than those of non-conjugants appears to furnish experimental proof of this idea.

Whatever be the true significance of conjugation and the cause of the many infertile syzygies that have been noted not only in these experiments but also by other investigators working on other species, the results here obtained show that the death of the ex-conjugants was not due, as far as could be told by the study of both living and prepared material, to low vitality previous to conjugation or to abnormal conditions at the time of conjugation, and the evidence derived from the present study points to the conclusion that one of the chief factors in producing infertile syzygies is an identity of the environmental history of the gametes.

In the experiments recorded in this paper it has been shown that descendants from the same original animal act differently with regard to conjugation when kept in different media, even though the other features of their environment be the same. In the stock of the Sb culture kept on the beef medium conjugation occurred at the 350th generation after having been kept on this medium for about four months. In a sub-culture, isolated from this one at the 150th generation and kept on a hay infusion medium, no conjugation ever occurred and this culture at the time it died out had been under observation altogether eleven months and had passed through a total of 572 generations. Again in a sub-culture isolated from the hay sub-culture and placed on the beef medium, conjugation occurred in the stock some three and one-half months later, after the culture had passed through 120 generations on the

beef. In both of the epidemics which occurred in the cultures kept on the beef medium it was very evident that the phenomenon was one which affected not merely a few individuals, but that it was an epidemic in the true sense of the word, and that conjugation would occur in the cultures during the epidemic whenever opportunity was afforded. In fact the only way by which it was possible to prevent conjugation was by the daily isolation of a single individual such as was done in the main lines of the cultures. Inasmuch as conjugation never occurred at any time in the hay culture, even though in both the length of time it was kept and the number of generations through which it passed it exceeded either of the cultures kept on the beef medium, there is conclusive evidence that neither the age of the organisms nor the number of the generations through which they passed were potent in inducing conjugation in these cultures. *The determining feature was the medium used and the results here recorded give definite evidence that the 'same protoplasm' under the influence of different culture media may show fundamental differences in its life history.*

The fact that the Sbh culture kept on the hay medium did not continue to live indefinitely does not show that the death of the culture was due to the ending of any definite life cycle. Maupas believed that 316 generations were the maximum number that *S. pustulata* could attain. If, as in this experiment, this number can be raised to 572 generations, there seems to be good reason for believing that, under other conditions of food and environment, the number can be raised still higher and it is probable that, under some conditions, they can be bred indefinitely. The work of Woodruff ('11b) with *Paramecium caudatum* appears to substantiate this view. He found that this organism died out in the culture in which fresh medium was supplied daily but that sister cells kept under other conditions continued to thrive, thus showing that the death of the culture in the one case was not due to the ending of any definite life-cycle, but to the fact that the environment supplied was not exactly adapted to the continued existence of this form. Other work by the writer, which is now in progress and will be reported in a later paper, shows the same result.

In the cultures kept on the beef medium, the results of the experiments show that a condition different from that in the hay culture was present. The cultures died out quite abruptly following the epidemics of conjugation. A study of both the living and the prepared material previous to the occurrence of conjugation entirely failed to reveal any pathological conditions present in the animals and indicated that the beef medium furnished a favorable environment for this race of *S. pustulata*. However, since in both the beef cultures all of the animals reached a physiological state in which conjugation was a necessity, as shown by the death and degeneration of the non-conjugants, it cannot be said that the beef medium was one which was suitable for the indefinite existence of this species without conjugation and therefore, in this sense, the medium was an unfavorable one for this species, since, as shown by Woodruff in his work with *Paramaecium aurelia*, conjugation is not a necessity for the continued and indefinite existence of protozoan protoplasm when a medium which is entirely suitable is supplied.

In brief, then, it is believed that the death of the cultures whether kept on a hay or a beef medium was not due to the ending of any definite life cycle but rather to the failure of either of these media to furnish an environment which was exactly suitable for the unlimited reproduction of this race of *Stylonychia pustulata*.

VIII. SUMMARY

1. The objects of these experiments were (1) to observe the effect of the different culture media upon the life history of *Stylonychia pustulata*, and (2) in case conjugation occurred to study its effect upon the progeny under the influence of the different media.

2. Two media were used in this work, viz., a 'constant' medium consisting of a 0.025 per cent solution of Liebig's extract of meat and a hay infusion medium.

3. Three cultures of *S. pustulata* have been under observation, all of which were started from descendants of an original individual isolated from a laboratory culture September 21, 1910. Culture Sb was started October 2, 1910, and was carried on a beef ex-

tract medium until February 24, 1911, when it died out at the 403rd generation, following an epidemic of conjugation. Culture Sbh was started November 23, 1910, from culture Sb when it was at the 150th generation and was carried on a hay infusion medium until August 15, 1911, when it died out at the 572nd generation. Culture Sbhb was started from culture Sbh March 3, 1911, at the 360th generation and was carried on the beef medium until July 5, 1911, when it died out at the 507th generation, following an epidemic of conjugation. Graphs showing the life history of each culture have been plotted by averaging the divisions in the four lines of each culture for ten day periods.

4. In the culture kept on the hay medium conjugation never occurred and abnormal or degenerating animals did not appear, but after a gradual decline in the fission rate the culture finally died out. It is not believed that this result was due to the ending of any definite life-cycle but rather to the failure of the hay medium to furnish an environment which was exactly suitable for the continued existence of this species. This view is substantiated by work to be reported in a subsequent paper.

5. In both of the cultures kept on the beef medium an epidemic of *infertile* conjugation occurred, and within three weeks after its appearance all the non-conjugants in both of the cultures died.

6. Careful study of the living and prepared material shows a normal morphological condition of the animals at the time of conjugation, but shortly after its appearance degenerative changes were evident.

7. All ex-conjugants passed through degenerative stages which ended in their death within forty-eight hours after separation without division.

8. A study of the prepared material shows that the micronuclei are present in non-conjugants, conjugants and ex-conjugants at all stages.

9. There is no evidence to show that the infertility of conjugation was due to any abnormal conditions previous to, or during, conjugation.

10. The experiments show conclusively, it is believed, that conjugation is induced by *external* conditions affecting the organism,

and that it bears no relation, in this form at least, to a particular period of a 'life-cycle.'

11. It is suggested that the infertility of the syzygies in these cultures is the result of the fact that the gametes had an identical environmental history.

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PLATE 1

EXPLANATION OF FIGURES

These photographs were taken from permanent preparations stained with picrocarmin. The same magnification was used in all cases and, therefore, the relative sizes represent absolute differences in the size of the different individuals.

1 A typical, normal individual from the beef cultures. This specimen was taken from the Sbhb culture at the 480th generation just prior to the June epidemic of conjugation.

2 to 6 These figures show non-conjugating animals in different stages of degeneration. They were preserved at different times during the February epidemic in the Sb culture. The cytoplasmic structure shows great irregularities. The micronuclei are to be seen in all the individuals but they are considerably enlarged and shifted from their normal condition. The macronuclei have lost their characteristic shapes. Figure 6 shows a very advanced stage of degeneration.

7 to 8 Two individuals from the Sb culture kept on the hay infusion medium. Figure 7 was preserved at the time of the February epidemic in the Sb culture and was at the 321st generation. Figure 8 is an individual preserved shortly before the death of the Sb culture at the 510th generation. No degeneration can be noted. One of the micronuclei can be seen in mitosis. The other micronucleus is in the same stage but is out of focus.

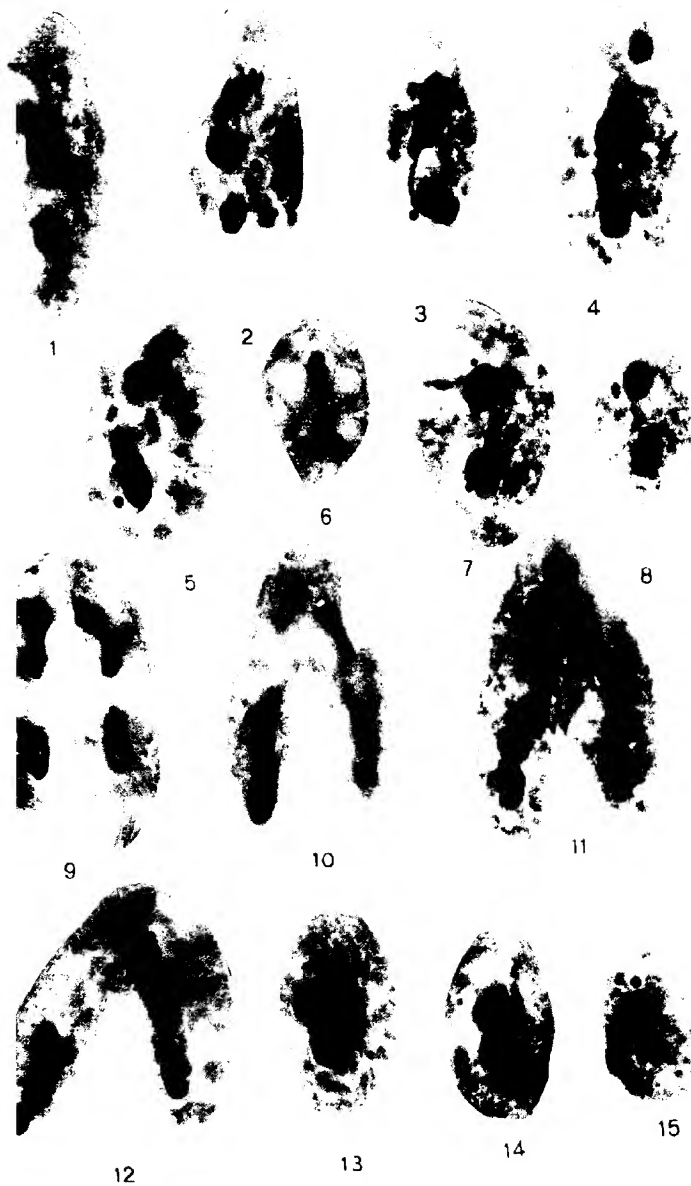
9 A pair of conjugants preserved during the June epidemic. This represents an early stage in conjugation before great change has taken place in the nuclear structure.

10 This pair of conjugants was preserved during the February epidemic and shows a somewhat later stage than figure 9. In the animal to the left can be seen the two micronuclei in the spindle stage of mitosis just previous to the first maturation division. The same stage is present in the other animal but is out of focus.

11 A pair of conjugants preserved during the February epidemic. In the animal to the right can be seen what is interpreted as a copulation nucleus. The same stage is out of focus in the other animal.

12 A pair of conjugants preserved during the February epidemic. This is a stage just previous to the separation of the gametes.

13 to 15 Three typical specimens of ex-conjugants preserved during the February epidemic. Figure 13 is a stage shortly after separation. Figure 14 shows a stage in which degeneration has begun and in figure 15 an individual well advanced in degeneration is shown.



DATA FOR THE STUDY OF SEX-LINKED INHERITANCE IN DROSOPHILA

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In recent years a new fact in Mendelian inheritance has come to light, which while it obscures the Mendelian expectation based on *independent* segregation of the factors of inheritance, shows that the main Mendelian principles are by no means invalidated; or, they too are manifest, but obscured by the linkage or coupling of certain factors. When certain somatic characters are associated with sex, as in *Drosophila*, we have the best opportunity, as yet afforded, for studying in its simplest form this sort of 'associative' inheritance; for, in certain combinations, the relation between linkage and breaking of the linkage ('crossing-over' as we shall call it) is shown at once by the male flies which indicate without complication the kinds of eggs that the F_1 female produces. In certain combinations both males and females give the same result. Such cases are those in which the sperm of the F_1 generation contains only sex-linked recessive or 'absent' characters.

In the following account we shall describe certain experiments in which three linked characters (in addition to sex) are involved; namely, red eyes, versus no red or white eyes; the black factor for body color (giving black or gray flies), versus its absence (which gives yellow or brown flies); and the factor for long wings versus the absence of that factor (which gives miniature wings). These characters show various strengths of linkage, i.e., the number of times any two of them hold together differs for each combination. This relation will be discussed after the data have been given.

Since these sex-linked factors follow the distribution of the sex-chromosomes we may think of them as contained in these chromo-

somes (X) when present, or absent from the chromosomes when the absence of the factor is involved. In the male-producing sperm, where no X is present, the sex-linked characters are always absent. A corollary to this point of view involves the crucial point of the chromosome theory of linkage (Morgan, '11). In the female two X-chromosomes are always present. If one of them contains two of the factors in question, such as the factor for red¹ (R) and that for long wings (L), and the other X-chromosome contains no factor for red (absence of red or W) and short wings (or S) it is possible for interchange (or 'breaking,' or 'crossing-over') between RL and WS to take place. How often this may occur depends on the strength of the linkage of the factors involved (which one of us has tried to interpret as due to their position in the chromosomes). But in the male, on the other hand, no such interchange is theoretically possible, and the results show that none occurs. It is this simple and consistent relation that gives 'point' to the chromosomal interpretation.

In order to have a basis for interpreting the more complicated cases we will first give the results of an experiment where only two contrasted characters, black and yellow, are involved. In this experiment no question of linkage is involved, since the factor for black and that for yellow are not in the same chromosome. The experiment also gives information showing the viability of the gray, black, yellow and brown flies.

When black females were mated to yellow males all the offspring were gray (N). These inbred gave the following results:

	LRB ♀ by LRY ♂	
F ₁	LRN ♀	50
	LRN ♂	60
F ₂	LRN ♀	316
	LRN ♂	153
	LRB ♀	104
	LRB ♂	48
	LRY ♂	164
	LRBr ♂	40

¹ In reality the presence of C gives red and its absence c white

The analysis is as follows:

		yBBrX - yBBrX	Black ♀	
		YbBrX - YbBr	Yellow ♂	
F ₁		yBBrXYbBrX	Gray ♀	
		yBBrXYbBr	Gray ♂	
Gametes	F ₁	YBBrX - yBBrX - YbBrX - ybBrX	Eggs	
		YBBrX - yBBrX - YbBr - ybBr	Sperm	
		YBBrXYBBrX	Gray ♀	YBBrXYbBr Gray ♂
		YBBrXyBBrX	Gray ♀	YBBrXybBr Gray ♂
		yBBrXYBBrX	Gray ♀	yBBrXYbBr Gray ♂
		yBBrXyBBrX	Black ♀	yBBrXybBr Black ♂
F ₂		YbBrXYBBrX	Gray ♀	YbBrXYbBr Yellow ♂
		YbBrXyBBrX	Gray ♀	YbBrXybBr Yellow ♂
		ybBrXYBBrX	Gray ♀	ybBrXYbBr Yellow ♂
		ybBrXyBBrX	Black ♀	ybBrXybBr Brown ♂
		Gray ♀ 6		Gray ♂ 3
		Black ♀ 2		Yellow ♂ 3
Summary of F ₂				Black ♂ 1
				Brown ♂ 1

The numbers fairly approximate to expectation. The reciprocal cross is as follows:

		LRB ♂ by LRY ♀	
F ₁		LRN ♀ = 89	
		LRY ♂ = 59	
		LRN ♀ = 212	
		LRN ♂ = 197	
		LRB ♀ = 79	
		LRB ♂ = 69	
F ₂		LRY ♀ = 181	
		LRY ♂ = 188	
		LRBr ♀ = 44	
		LRBr ♂ = 71	

The analysis follows:

		YbBrX - YbBrX	Yellow ♀	
		yBBrX - ybBr	Black ♂	
F ₁		YbBrXyBBrX	Gray ♀	
		YbBrXybBr	Yellow ♂	

Gametes F ₁	ybBrX - YbBrX - yBBrX - YBBrX	Eggs
	ybBrX - YbBrX - ybBrX - YbBr	Sperm
Summary of F ₂	Gray ♀ 3	Gray ♂ 3
	Black ♀ 1	Black ♂ 1
	Yellow ♀ 3	Yellow ♂ 3
	Brown ♀ 1	Brown ♂ 1

The results agree again fairly well with the expectation. The numbers of gray males and females are quite close. This correspondence between the sexes holds throughout, although the females exceed the males in the grays and blacks, while the males are in excess in the yellows and browns.

In the next case also the same two characters are involved, viz., yellow and black, but combined with short (miniature) wings and white eyes. The analysis is the same as in the last case, and need not be repeated:

	SWB ♀ by SWY ♂
F ₁	SWN ♀ = 78
	SWN ♂ = 62
F ₂	SWN ♀ = 355
	SWN ♂ = 173
	SWB ♀ = 111
	SWB ♂ = 44
	SWY ♂ = 149
	SWBr ♂ = 42

The reciprocal cross was also made:

	SWY ♀ by SWB ♂
F ₁	SWY ♂ = 88
	SWN ♀ = 69
F ₂	SWN ♀ = 68
	SWN ♂ = 71
	SWB ♀ = 10
	SWB ♂ = 11
	SWY ♀ = 38
	SWY ♂ = 64
	SWBr ♀ = 12
	SWBr ♂ = 11

CROSSES INVOLVING TWO SEX-LINKED CHARACTERS

In the following crosses black and yellow body-color, white and red eyes are involved. In these cases there are only two sex-linked factors, viz., the factor for black and the factor for red (and their respective absences). In reality, it is the color-producer, C, and not red color, R, that is the factor involved in the eye-color inheritance; but with this explanation no misunderstanding will arise if we use the symbol R for red eye and W for its absence.

In the first case long winged, white eyed, black females were crossed with long winged, red eyed, yellow males. If all the female classes were realized in F_2 there would be many more classes than actually appear, but owing to the linkage between W and B from the grandmother, and R and b from the grandfather there will be only eight large classes. The smaller classes represent the breaking of the linkage or 'crossing-over:'

LRY σ^7 by LWB φ

F_1 LRN φ = 244
 LWN σ^7 = 253

F_2 LRN φ = 1549
 LRB φ = 490
 LWN φ = 1120
 LWN σ^7 = 1283
 LWB φ = 368
 LWB σ^7 = 451
 LRY σ^7 = 1042
 LRBr σ^7 = 217

 LRN σ^7 = 3
 LWY σ^7 = 1
 LRB σ^7 = 1

The analysis is as follows. The factor for Brown (Br) is here omitted:

	WByX - WByX'	White black ♀
	RbYX - WbY	Red yellow ♂
F ₁	WByXRbYX WByXWbY	Red gray ♀ White gray ♂
Gametes of F ₁	WBYX - WByX - RbYX - RbyX WBYX - WByX - WbY - Wby	Eggs Sperm
F ₂	Gray white ♀ 3 Gray red ♀ 3 Black white ♀ 1 Black red ♀ 1	Gray white ♂ 3 Yellow red ♂ 3 Black white ♂ 1 Brown red ♂ 1

There were five cases of crossing-over of color factors in the males, in a total of 2993 males; approximately, once in 600 times. The crossing-over was between Rb and WB. Each time that the crossing-over occurs one way, there should be on an average a counter-cross the other way. Thus, when an interchange between Rb and WB takes place the combination RB will occur as often as Wb. We should expect, therefore, to find a balance in the results, except in so far as accident or death obscure the output. In the present case the cross-over RB survived four times (giving 3, LRN and 1, LRB), while the counter-cross Wb survived only once. Three of the counter-crosses are not represented. The result can probably be explained by the lower viability that exists for the Yellow-whites.

The reciprocal cross is given below. There were also eight large classes but no cases of crossing-over. The numbers are much fewer than in the last case.

	LRY ♀ by LWB ♂
F ₁	LRN ♀ = 255 LRY ♂ = 262
F ₂	LRN ♀ = 111 LRB ♀ = 27 LWN ♂ = 92 LWB ♂ = 15 LRY ♀ = 71 LRY ♂ = 127 LRBr ♀ = 10 LRBr ♂ = 26

The analysis is as follows:

	RbYX - RbYX	Red yellow ♀
	WByX - Wby	White black ♂
F ₁	RbYXWByX	Red gray ♀
	RbYXWby	Red yellow ♂
Gametes of F ₁	RbyX - RbYX - WByX - WBYX	
	RbyX - RbYX - WbY - Wby	
F ₂	Brown red ♀ 1	Brown red ♂ 1
	Yellow*red ♀ 3	Yellow red ♂ 3
	Black red ♀ 1	Gray white ♂ 3
	Gray red ♀ 3	Black white ♂ 1

The result conforms fairly with expectation; the Yellow red running behind.

In the next crosses a third combination was present. Six large classes are expected.

	LRB ♀ by LWY ♂	
F ₁	LRN ♀ = 57	
	LRN ♂ = 48	
	LRN ♀ = 298	
	LRN ♂ = 158	
F ₂	LRB ♀ = 75	
	LRB ♂ = 54	
	LWY ♂ = 88	
	LWBr ♂ = 37	
	LWN ♂ = 5	

The analysis follows:

	RByX - RByX	Red black ♀
	WYbX - WYb	White yellow ♂
F ₁	RByXWYbX	Red gray ♀
	RByXWYb	Red gray ♂
Gametes of F ₁	RBYX - RByX - WYbX - WybX	
	RBYX - RByX - WYb - Wyb	
F ₂	Gray red ♀ 6	Gray red ♂ 3
	Black red ♀ 2	Black red ♂ 1
		Yellow white ♂ 3
		Brown white ♂ 1

There were five cases of crossing-over (males) in a total of 337 males. The cross-overs are all of one kind and result from the combination of W and B. The return crosses would be Red-yellows and these did not appear. The Red-yellows are, in fact, less viable than the White-blacks.

In the reciprocal cross eight large classes are expected. Only two classes of crossing-over were found. This again indicates a close union between R and B:

	LRB σ^7 by LWY φ	
F ₁	LRN φ = 198	
	LWY σ^7 = 143	
F ₂	LRN φ = 341	
	LRN σ^7 = 285	
	LRB φ = 153	
	LRB σ^7 = 84	
	LWY φ = 253	
	LWY σ^7 = 229	
	LWBr φ = 61	
	LWBr σ^7 = 61	
	LWN σ^7 = 6	
	LWB σ^7 = 3	

The analysis follows:

	WbYX - WbYX	White yellow φ
	RByX - Wby	Red black σ^7
F ₁	WbYXRByX	Red gray φ
	WbYXWby	White yellow σ^7
Gametes of F ₁	WbyX - WbYX - RByX - RBYX	
	WbyX - WbYX - Wby - WbY	
F ₂	Brown white φ 1	Brown white σ^7 1
	Yellow white φ 3	Yellow white σ^7 3
	Black red φ 1	Black red σ^7 1
	Gray red φ 3	Gray red σ^7 3

There were nine cases of crossing-over, all males, in 659 males and 818 females. In this cross since all the F₁ sperm, both female and male-producing, carry only recessive sex-linked factors,

both sexes count in the ratio. This gives 9 to 1477 or 1 to 164. These cases are all in one direction. The missing counter-crosses should be Red-yellows.

The remaining cross involves the same combinations as one of the preceding, but the R and B factors are present in the gray fly. Therefore no crossing-over would be visible. Six large classes are expected:

LWB ♂ by LRN ♀	
F ₁	LRN (intermediate) ♀ and ♂ = 1085
	LRN ♀ = 2714
	LRN ♂ = 1541
	LWN ♂ = 1033
F ₂	LRB ♀ = 793
	LRB ♂ = 330
	LWB ♂ = 305

The analysis follows:

	RBYX — RBYX	Red gray	♀
	WByX — Why	White black	♂
F ₁	RBYXWByX	Red gray	♀
	RBYXWby	Red gray	♂
Gametes of F ₁	RByX — RBYX — WByX — WBYX		
	RByX — RBYX — Why — WbY		
	Gray red ♀ = 6	Gray red ♂ = 3	
	Black red ♀ = 2	Black red ♂ = 1	
F ₂		Gray white ♂ = 3	
		Black white ♂ = 1	

In the last case no evidence of crossing-over in the eye factors is expected; for, the normal and black flies are alike in that they carry the sex-linked factor for black. The yellow factor, it is true, is absent from Black and present in Normal, but it shows no linkage with White or with Red, which is in accord with the hypothesis here followed.

In the reciprocal cross eight large classes are expected, which occur. No crossing-over was expected or rather could have been observed even if it occurred:

LWB ♀ by LRN ♂	
F ₁	LWN (intermediate) ♀ 16
	LRN (intermediate) ♂ 15
F ₂	LRN ♀ = 1647
	LRN ♂ = 1327
	LRB ♀ = 449
	LRB ♂ = 381
	LWN ♀ = 1171
	LWN ♂ = 1280
	LWB ♀ = 379
	LWB ♂ = 375

THE HEREDITY OF THREE CONTRASTED SEX-LINKED CHARACTERS

In the following crosses three contrasted sex-linked factors are involved, one for wings, one for eye color, and one for body color.

The factors involved are the same as those of the preceding crosses. Long wings (L) or normal wings contrast with miniature wings (S), recessive to the former. Two factors, both sex-linked, give, when both present, long wings, while miniature wings are due to the absence of one of them; the absence of the other producing the wing called rudimentary, but for simplicity the letters L and S may be used, if one remembers that L is in the X chromosome, and therefore present only in the female-producing sperm; while S merely means the absence of L, and alone stands for miniature wings.

The factor for red eyes (R) is also sex-linked (in the sense that the factor C is present); the absence of red is white eyes (W), which means that the factor C is absent; small c would express this condition more logically, but less graphically.

The black factor B is also sex-linked, and present, therefore, only in the female-producing sperm. Its absence is represented by small b. The factor for yellow (Y) is not sex-linked, and,

therefore, does not follow X in its distribution. Its absence is indicated by small y. In all there were five crosses with their reciprocals, or a total of ten combinations.

{	SRB	♀	by	LWY	♂
{	SRB	♂	by	LWY	♀
{	LWB	♀	by	SRY	♂
{	LWB	♂	by	SRY	♀
{	SWB	♀	by	LRY	♂
{	SWB	♂	by	LRY	♀
{	LRB	♀	by	SWY	♂
{	LRB	♂	by	SWY	♀
{	SRB	♀	by	LWN	♂
{	SRB	♂	by	LWN	♀

Three combinations, viz: the second, third and fourth gave some anomalous results and have been withdrawn in order that they may be repeated.

In representing the gametes of F_1 we have followed the plan of writing in the upper line of 'Eggs,' in the two middle terms, the two combinations that come direct from the paternal and maternal gametes, and at their sides, right and left, the eggs that come from the free distribution of Y and y. In the second line of eggs, written in similar sequence, are the crossing-over of long and short. The crossing of RB and Wb (or similar combinations) is not given, but can be readily conceived. It is the latter that gives the small classes of crossing-over for color (eye and body color) which follow the numerical data of the larger classes. The sperms are given in the third line of gametes and since by the hypothesis here followed no crossing-over in the gametes of the males is allowable, only one line (of four classes) is represented. In the sperm the yellow factor freely interchanges, since it shows no linkage with the sex-factor X. The results fully justify this assumption. Instead of writing out all the combinations of egg and sperm, a summary only of the expected results is given at the end of the analyses.

SHORT, RED, BLACK BY LONG, WHITE, YELLOW

When the female, SRB is mated to the male LWY all the female offspring are long, red, gray; and the males are short, red, gray. The results for the next F_2 generation are given below:

SRB ♀ by LWY ♂	
F_1	LRN ♀ 98
	SRN ♂ 80
F_2	LRN ♀ = 523
	LRN ♂ = 146
	LRB ♀ = 110
	LRB ♂ = 31
	SRN ♀ = 292
	SRN ♂ = 242
	SRB ♀ = 53
	SRB ♂ = 82
	LWY ♂ = 216
	LWBr ♂ = 40
	SWY ♂ = 85
	SWBr ♂ = 30
	LWN ♂ = 2
	LRY ♂ = 1
	SRY ♂ = 3
	SWB ♂ = 1

The expectation is:

SRByBrX - SRByBrX		Short red black	♀
LWYbBrX - SWYbBr		Long white yellow	♂
F_1	SRByBrXLWbYBrX	Long, red, Gray	♀
	SRByBrXSWbYBr	Short, red, Gray	♂
Gametes of F_1	{ SRBYBrX - SRByBrX - LWbYBrX - LWbyBrX } Eggs		
	{ LRBYBrX - LRByBrX - SWbYBrX - SWbyBrX } Sperm		
{ SRByBrX - SRBYBrX - SWbYBr - SWBYBr }			

L or SRN ♀ 6	S or LWY ♂ 3
L or SRB ♀ 2	S or LWBr ♂ 1
	L or SRN ♂ 3
	L or SRB ♂ 1

There were seven cases of crossing-over in color, all males, in a total of 872 males, or 1 to 97. Of these seven, three represent one crossing-over (LWN and SWB), and four the counter-cross. In this instance the balance is held despite the lesser viability of the Red-yellows.

The crossing-over of long, L, (normal) and short, S, (miniature) is shown in the large classes. The linkage is so 'loose,' that these two characters appear almost as though no linkage existed, but an examination shows that where S is combined with RB, and L with Wb the classes of SRB are relatively larger than those of LRB; while conversely the LWY classes are larger than the corresponding SWY. A comparison of the records with the expectation makes this evident at once. It is most apparent in the males, where no complications exist. Thus LWY ♂ = 216, while SWY ♂ = 85; LWB ♂ = 40, while SWB ♂ = 30, and on the other hand SRN ♂ = 242, while LRN ♂ = 146; and the SRB ♂ = 82, while LRB ♂ = 31. The sum of the 'straight' males is 580, while that of the cross-overs is 292. The gametic ratio is therefore 2:1.

SHORT, WHITE, BLACK BY LONG, RED, YELLOW

When the female is SWB and the male LRY the offspring are LRN ♀ and SWN ♂. The results in the next generation are given below:

	SWB ♀ by LRY ♂	
F ₁	LRN ♀	228
	SWN ♂	181

direction. The recessive RB combination may be obscured by the red, normal or black, female classes.

Concerning the cases of crossing-over for character of wing SWN $\sigma = 418$, while LWN $\sigma = 224$; SWR $\sigma = 145$, while LWB $\sigma = 95$. On the other side, LRY $\sigma = 419$, while SWY $\sigma = 161$; LRBr $\sigma = 102$, while SRBr $\sigma = 47$. The sum of the 'straight' males is 1081, and that of the cross-overs 527; or almost exactly 2 to 1.

The reciprocal cross is as follows:

	SWB σ by LRY φ
	LRN $\varphi = 121$
	LRY $\sigma = 76$
F_2	LRN $\varphi = 808$
	LRB $\varphi = 215$
	LWN $\sigma = 191$
	LWB $\sigma = 63$
	SWN $\sigma = 221$
	SWB $\sigma = 108$
	LRY $\varphi = 442$
	LRY $\sigma = 335$
	LRBr $\varphi = 76$
	LRBr $\sigma = 73$
	SRY $\sigma = 118$
	SRBr $\sigma = 17$

The analysis follows:

P ₁		LRYbX	—	LRYbX	
		SWByX	—	...yb	
Gametes of F ₁	LRbyX	—	LRYbX	—	SWByX
	SRbyX	—	SRYbX	—	LWByX
	LRbyX	—	LRYbX	—	...yb
					...Yb
					Sperm
F ₂		LRBr	♀ = 2	LRBr	♂ = 1
		LRY	♀ = 6	SRBr	♂ = 1
		LRB	♀ = 2	LRY	♂ = 3
		LRN	♀ = 6	SRY	♂ = 3
				SWB	♂ = 1
				LWB	♂ = 1
				SWN	♂ = 3
				LWN	♂ = 3

Despite the large number of individuals (2677), there are no cases of crossing-over for color in the last case. If such occurred in the females, they would be masked by the presence of the dominant factor, R, present in all female-producing sperm. Concerning the crossing-over of wing characters, it will be noted that LRY $\sigma^s = 335$, while SRY $\sigma^s = 118$; and LRBr $\sigma^s = 73$, while SRBr $\sigma^s = 17$; and the on other hand, SWB $\sigma^s = 108$, while LWB $\sigma^s = 63$ and SWN $\sigma^s = 221$, while LWN $\sigma^s = 191$. The sum of the 'straight' cases is 737, while that of the cross-overs is 398, or approximately 2 to 1.

LONG, RED, BLACK BY SHORT, WHITE, YELLOW

When LRB females are paired with SWY males, the offspring are LRN σ^s 's and φ 's. The second generation is given below:

	LRB φ by SWY σ^s	
F ₁	LRN φ	94
	LRN σ^s	111
F ₂	LRN φ	= 1819
	LRN σ^s	= 562
	LRB φ	= 463
	LRB σ^s	= 163
	LWY σ^s	= 279
	LWBr σ^s	= 78
	SWBr σ^s	= 194
	SWY σ^s	= 371
	SRN σ^s	= 266
	SRB σ^s	= 53
	LWN σ^s	= 3
	LRY σ^s	= 3
	LRBr σ^s	= 1
	(LRY φ	= 3)

The expectation is as follows:

P.	LRByX	—	LRByX
	SWYbX	—	...Yb

Gametes of F ₁	LRBYX - LRByX - SWYbX - SWybX	} Eggs
	SRBYX - SRByX - LWYbX - LWybX	
	LRBYX - LRByX - Y - Y	Sperm
F ₂	LRN ♀ 12	LRN ♂ 3
	LRB ♀ 4	SRN ♂ 3
		SWY ♂ 3
		LWY ♂ 3
		LRB ♂ 1
		SRB ♂ 1
		SWBr ♂ 1
		LRBr ♂ 1

There were seven cases of crossing-over in 1466 males, and three cases in 2282 females. In the males the three crossings in one direction (LWN) are balanced by four crossings in the other direction (LRY, and LRBr). The three cases in the females (LRY) can not be explained by crossing-over in the eggs, since all female-producing sperms carry the factor for black, hence no yellow females are possible, unless one of these sperms should lose the B factor, which would be a mutation. Hence the case is due either to this, or to some error.

In the wing characters we find LRN ♂ = 562, while SRN ♂ = 266; LRB ♂ = 163, while SRB ♂ = 53. On the other hand, SWY ♂ = 371, while LWY ♂ = 279; SWBr ♂ = 184, while LWBr ♂ = 78. The sum of the 'straight' males is 1280, and the cross-overs 676, a ratio of about 2 : 1.

The reciprocal cross gave the following results:

		LRB ♂ by SWY ♀	
P ₁	LRN ♀	83	
	SWY ♂	79	
F ₂	LRN ♀	= 355	
	LRN ♂	= 301	
	LRB ♀	= 90	
	LRB ♂	= 90	
	LWY ♀	= 164	
	LWY ♂	= 116	
	LWBr ♀	= 35	
	LWBr ♂	= 36	
	SWY ♀	= 147	
	SWY ♂	= 174	
	SWBr ♀	= 50	
	SWBr ♂	= 48	
	SRN ♀	= 140	
	SRN ♂	= 150	
	SRB ♀	= 41	
	SRB ♂	= 36	
	SWN ♂	= 2	
	LWN ♂	= 5	
	LRY ♂	= 3	
	SWN ♀	= 2	
	LWN ♀	= 9	(in one bottle)

The expectation is as follows:

P ₁	SWYbX - SWYbX			
	LRByX - ...by			
Gametes of F ₁	SWbyX - SWbYX - LRByX - LRBYX	} Eggs		
	LWbyX - LWbYX - SRByX - SRBYX			
F ₂	SWbyX - SWYbX - ...by - ...bY	Sperm		
	SWB ♀ = 1	SWBr ♂ = 1		
	LWB ♀ = 1	LWBr ♂ = 1		
	SWY ♀ = 3	SWY ♂ = 3		
	LWY ♀ = 3	LWY ♂ = 3		
	LRB ♀ = 1	LRB ♂ = 1		
	SRB ♀ = 1	SRB ♂ = 1		
	LRN ♀ = 3	LRN ♂ = 3		
	SRN ♀ = 3	SRN ♂ = 3		

There were ten cases of crossing-over in 951 males, and eleven cases in 1022 females. In the former there were seven cases in one direction (SWN and LWN) balanced by only three cases in the opposite direction (LRY); but the latter are known to be less viable. In the females the eleven cases were in the same direction (SWN and LWN). Of these nine occurred in one bottle, suggestive of some error.

In wing characters there were LRN ♂ 301, while SRN ♂ = 150; LRB ♂ = 90, while SRB ♂ = 36; on the other hand, SWY ♂ = 174, while LWY ♂ = 116; SWBr ♂ = 48, while LWR ♂ = 36. The sum of the 'straight' males is 613, and the cross-overs 332, approximately 2 to 1.

SHORT, RED, BLACK BY LONG, WHITE, NORMAL

When females SRB are mated to LWN, the female offspring are LRN, and the males SRN. The second generation is given below:

SRB ♀ by LWN ♂		
F ₁	LRN ♀	195
	SRN ♂	147
F ₂	LRN ♀	= 885
	LRN ♂	= 316
	LRB ♀	= 215
	LRB ♂	= 94
	LWN ♂	= 410
	LWB ♂	= 130
	SRN ♀	= 482
	SRN ♂	= 572
	SRB ♀	= 151
	SRB ♂	= 198
	SWN ♂	= 166
	SWB ♂	= 51

The expectation is as follows:

P ₁	SRByX	—	SRByX
	LWBYX	—	...bY

Gametes of F_1	SRBYX	-	SRByX	-	LWBYX	-	LWByX	} Eggs
	LRBYX	-	LRByX	-	SWBYX	-	SWByX	
	SRBYX	-	SRByX	-	...bY	-	...by	Sperm

F_2	SRN	♀ 6	SRN	♂ 3
	LRN	♀ 6	LRN	♂ 3
	SRB	♀ 2	LWN	♂ 3
	LRB	♀ 2	SWN	♂ 3
			SRB	♂ 1
			LRB	♂ 1
			LWB	♂ 1
			SWB	♂ 1

There was no evidence of crossing of color factors in 1937 males, and none are expected, since both Black and Normal carry the black factor; and the yellow factor that distinguishes them is not sex-linked. In wing characters SRN ♂ = 572, while LRN ♂ = 316, SRB ♂ = 198, while LRB ♂ = 94. On the other hand, LWN ♂ = 410, while SWN ♂ = 166; LWB ♂ = 130, while SWB ♂ = 51. The sum of the 'straight' males is 1310 and the cross-overs 627, nearly 2 to 1.

The reciprocal cross is as follows:

SRB ♂ by LWN ♀

F_1	LRN	♀	514
	LWN	♂	404

F_2	LRN	♀	= 831
	LRN	♂	= 312
	LRB	♀	= 278
	LRB	♂	= 78
	LWN	♀	= 661
	LWN	♂	= 616
	LWB	♀	= 189
	LWB	♂	= 184
	SRN	♂	= 282
	SRB	♂	= 146
	SWN	♂	= 244
	SWB	♂	= 89
	(SWN	♀	= 5) (in one bottle)

The expectation is as follows:

		LWBYX - LWBYX			
P ₁		SRByX - ...by			
Gametes of F ₁	LWByX - LWBYX - SRByX - SRBYX				} Eggs
	SWByX - SWBYX - LRByX - LRBYX				
	LWByX - LWBYX - ...by - ...Y				Sperm
		LWB ♀ 2	LWB ♂ 1		
		LWN ♀ 6	SWB ♂ 1		
		LRB ♀ 2	LWN ♂ 3		
		LRN ♀ 6	SWN ♂ 3		
F ₂			SRB ♂ 1		
			LRB ♂ 1		
			SRN ♂ 3		
			LRN ♂ 3		

There were 5 SWN ♀'s, all from one bottle; which can not be explained by crossing-over. They are either mutations or due to error.

Concerning wing-characters, SRB ♂ = 146, while LRB ♂ = 78; SRN ♂ = 282, while LRN ♂ = 312. On the other hand, LWN ♂ = 616, while SWN ♂ = 244; LWB ♂ = 184, while SWB ♂ = 89. The sum of the 'straight' males is 1228, while that of the cross-overs is 721. The 'straights' are less than twice the cross-overs, due largely to the SRN's being actually less than the LRN's.

CONCLUSION

The principal object of the preceding experiments was to study the phenomenon of linkage on a relatively large scale. The results show that whatever sex-linked factors enter into combination, those factors come out together in the grandchildren. Moreover, it makes no difference from the point of view of linkage whether a 'present' factor is linked to another 'present' factor or to an 'absence,' or even whether two absences are linked together in one of the parents. The phenomenon is the same, as both Bateson and I have pointed out.² The results are the more striking when

² This statement is too sweeping in so far as based on the evidence given in the text.

it is found in the same experiments that other factors not showing sex-linked inheritance do not show associative inheritance with those factors that are sex-linked. It is here that the chromosomal hypothesis seems to give an insight into the nature of the difference in the two cases.

In those counts where crossing-over in color is expected there were 61 such cases in a total of 12115; a gametic ratio of 1 to 198. The crossing-over is such a rare occurrence that although the total number of individuals is large, it is by no means large enough to make the ratio significant.

On the other hand, where the gametic ratio for crossing is low, as in the wing-characters, the numbers suffice to make the values significant, and it will be observed how closely these approximate 1 to 2. Moreover, the crossings balance quite well the counter-crossings, which is expected on the theory of chromosomal interchanges.

The same balance is expected for the color ratio but here we find, as the following table shows, that 18 crossings were in one direction and 43 in the other:

RB	Wb		
4	1		
0	0	in	260
WB	Rb		
5	0	in	337
9	0	in	1477
WB	Rb		
3	4	in	872
RB	Wb		
1	6	in	1611
0	0	in	1126
WB	Rb		
3	4	in	1466
1	3	in	1973
43	18		12115

But these numbers are, as stated, too small to be significant. It should be noted that no *actual* exchanges between the eggs them-

selves are expected, since one of the exchanges always goes into the polar body; but on an average the loss to the polar body should be as often of one kind as of the other. In order to test this more fully the 'exchanges' should be equally viable, which is not the case in the present experiments, where a correction for viability has to be supplied.

It will be noted that the few cases, in which impossible female classes appear, have been 'explained away' as due to mutation or to error. We believe that we are justified in making this assumption on the basis of our general knowledge of the behavior of these factors in heredity and of the possible experimental errors. But a critic will not be slow in pointing out that these cases can be explained if crossing-over is admitted for the male. The fairness of this criticism must be admitted. Whether it is justified, further work must show, and this work is under way.

STUDIES ON THE DYNAMICS OF MORPHOGENESIS AND INHERITANCE IN EXPERIMENTAL REPRODUCTION

IV. CERTAIN DYNAMIC FACTORS IN THE REGULATORY MORPHOGENESIS OF PLANARIA DOROTOCEPHALA IN RELATION TO THE AXIAL GRADIENT

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I. INTRODUCTORY

In the first paper of this series (Child, '11 c) attention was called to the existence of a gradient or gradients of some sort along the chief axis of the body of *Planaria dorotocephala*, as indicated by the regional differences in the regulation of pieces.

In the second paper (Child, '11 e) some evidence was presented for the view that the head region is the dominant region in mor-

phogenetic development as well as in function in the adult, and in the third paper (Child, '11 f) the process of asexual reproduction in *Planaria* was shown to be a necessary consequence of the existence of a distance limit in the dominance of the head region. Parts which come to lie beyond this limit, either in consequence of growth or of decrease in the effective distance of correlative factors originating in the dominant part (Child, '11 a) return to the fundamental reaction of planarian protoplasm, which is represented in morphogenesis by the formation of a head or the initial steps in this process, according to the degree of physiological isolation and the rate of the dynamic processes.

It was shown that in *Planaria* the failure of the posterior zooids to undergo visible morphogenetic development while still attached to the parent body is due to the fact that they are not completely isolated physiologically from the dominant head region of the animal.

The purpose of the present paper is to present certain data which throw light upon the problem of the nature of the axial gradient. These data concern certain features of the regulatory morphogenesis of pieces under experimental conditions.

II. EXPERIMENTAL DATA

A. THE AXIAL FACTOR IN REGULATION UNDER CONSTANT EXTERNAL CONDITIONS

In the first paper of this series the axial factor in regulation as it appears under the usual conditions of existence was considered at some length: the most important points of that consideration must be briefly stated here. They are as follows: first, the axial differences in rate consist of a decrease in the rate of regulation with increasing distance from the original anterior end; second, in longer pieces the size of the head and in shorter pieces the frequency of normal head-formation also decrease with change of level in the posterior direction; third; in all pieces the pharynx is farthest posterior in the most anterior pieces, i. e., the prepharyngeal region is longest in such pieces, and its length

decreases as the level of the piece becomes more posterior in the body; and finally the relative amount of regeneration in the stricter sense as compared with redifferentiation increases at the anterior end and decreases at the posterior end as the level of the piece becomes more posterior.

These statements hold good only for pieces within the limits of a single zoöid. With the passage from the posterior end of one zoöid to the anterior end of another there is a marked change in the axial factor or gradient: the pieces from the 'anterior' region of the second zoöid, for example, show more anterior characteristics than those from the posterior region of the first zoöid, which originally lay just in front of them (Child, '11 c, '11 f).

Of the four regional differences in regulation along the main axis which were mentioned above, the first, the rate difference, the third, the difference in length of the prepharyngeal region, and the fourth, the difference in proportion of regeneration and redifferentiation, are all obviously quantitative in character. The second regional difference as stated above consists in the longer pieces of a difference in the size of the head, also obviously a quantitative difference, but in the shorter pieces of a difference in the character of the head, which, according to some points of view, must be qualitative. As a matter of fact, these differences are also primarily quantitative, for it has been possible to produce experimentally all these axial differences in the character of the head by quantitative changes in external factors, e. g., temperature (Child, '11 d; Child and McKie, '11). Experiments of this sort will be considered in another connection.

In short, the differences in the regulation of similar pieces from different regions along the main axis indicate the existence of a quantitative gradient or gradients of some sort along the axis, but they do not give any evidence for the existence of qualitative axial differences, i.e., of a graded distribution of substances such as Morgan has at various times suggested.

It will be shown, however, in section C below and in later papers that the matter is not as simple as it appears from the comparison of pieces in sequence under constant conditions. As a matter of fact a metabolic gradient does exist along the main axis of the

body in the uninjured worm, but the differences in capacity for and rate of head-formation are related to this gradient only indirectly. Moreover, the rate of the dynamic processes in isolated pieces is not the same as before isolation and the effect of isolation on the rate of reaction differs in a characteristic manner for different levels of the body. The axial gradient which appears in the regulation of pieces taken in order along the axis is of course related to the dynamic axial gradient existing in uninjured worms, but as will appear more clearly later, the relation is not direct and immediate.

To sum up; the comparison of regulation of pieces from different levels along the axis and under constant external conditions indicates the existence of some sort of gradient or gradients in the uninjured animal, but gives us no direct evidence concerning the nature of the gradient.

B. THE AXIAL FACTOR IN THE REGULATION OF PIECES UNDER EXPERIMENTAL CONDITIONS

If an axial gradient does exist in the planarian body, such a gradient must either persist as a fraction of the gradient in the parent body or arise de novo in every piece that regulates into a new whole and the possibility exists of obtaining further evidence concerning the nature of the gradient with the aid of experimental conditions. For example, if the rate of reaction in morphogenesis at the posterior end of a piece of *Planaria* is appreciably less than that at the anterior end it should be possible with the aid of external factors which decrease the rate of reaction, to reduce the rate at the posterior end to such a level that morphogenesis would cease or almost cease there while it still went on at an appreciable rate in more anterior regions.

As a matter of fact it is possible to demonstrate the axial gradient in this manner with various external factors, e.g., the anesthetics alcohol, ether chlorotone, etc., low temperature, metabolic products in the water, potassium cyanide, lack of nutrition and other factors which decrease the rate of the reactions in the organism. The effects of all the conditions mentioned are

essentially similar: as the concentration of the anesthetic or other depressing agent increases, as the temperature becomes lower, as starvation advances, etc., my observations show that in all cases the processes at the posterior end of the piece are inhibited first and after these others in sequence toward the anterior end.¹ It is possible in fact, with proper conditions, to inhibit only tail-formation, or both tail-formation and pharynx-formation, or everything except head-formation, or finally head-formation itself.

Moreover, when we compare similar processes in pieces from different levels we find that they are not the same in all respects. In the case of head-formation itself, for example, quantitatively different conditions are necessary to produce a given effect on morphogenesis in pieces from different levels. In general abnormal heads are produced or head-formation is inhibited by less extreme conditions in pieces from the posterior region of the first zoöid than in similar pieces from the anterior region. Besides this the results differ characteristically at different levels of the body according to the way in which the depressing agent or condition is used. We can then obtain evidence concerning the relation between the regulation of pieces and the axial gradient in a variety of ways. Some lines of this evidence are presented in the following pages.

¹ The fact that under certain conditions pieces fail to form heads and yet give rise to long posterior outgrowths 'headless' pieces (Child, '11 c, '11 d) may seem to be a direct contradiction of this statement, but it is not. It was pointed out in the preceding paper (Child, '11 f, pp. 227-231) that the outgrowth at the anatomical posterior end of headless pieces is not physiologically simply a posterior end but is part of a new zoöid or series of zoöids and so possesses a higher rate of reaction than the regions of the old tissue anterior to it. In such cases it is the absence of a head that makes possible the establishment of a new head region in the posterior part of the piece. This new head region may be and usually is the region of highest rate of reaction in the piece and may dominate it (Child, '11 f, pp. 239-241). In the headless pieces, then, instead of a new tail, a new zoöid may arise at the posterior end of the piece and we find that the development and growth of the new zoöid up to a certain stage is less readily inhibited by external factors than is the formation of a tail.

On the other hand, the statement that under the increasing action of depressing factors the morphogenic processes are inhibited in sequence from the posterior end anteriorly is true only for pieces which consist of a single zoöid or part of a zoöid and in which the axial gradient is simple and continuous. For such pieces, however, it holds in all cases, so far as my observations go.

The influence of external factors on regulation or 'experimental reproduction' in *Planaria* will be considered more fully in later papers. At present we are concerned merely with evidence bearing upon the problem of the axial gradient. The data presented below show the effect of various external factors upon the morphogenic regulatory processes. They comprise a few characteristic series and their number might be greatly increased if it were necessary. But as regards the points to which attention is called these series do not differ in any essential respect from any others recorded in my notes, consequently no useful purpose is served by giving in full the records of large numbers of experiments. Since I have used anesthetics more extensively than other agents and conditions in experiments along the line at present under consideration, examples of the results obtained with anesthetics are given first and those with other external factors, which supplement and confirm them, follow.

1. Experiments with anesthetics

In these experiments the anesthetics were used in such dilution that they did not produce complete narcosis and death but usually allowed regulation to take place to a greater or less extent. The comparison of pieces from different levels and of different regions of the same piece shows, however, that a given concentration of the anesthetic may have a very different effect at different levels along the original axis or the axis of the piece.

The results obtained in the experiments described below are characteristic and are all confirmed by other series. The anesthetics were used in various ways: in my earliest experiments the mixture of the desired concentration was placed in a glass dish with ground edge and covered with a glass plate. Every twenty-four or forty-eight hours it was replaced by fresh mixture. Where quantitative results were not required this method was fairly satisfactory. But where different series were to be compared some better method of preventing decrease in concentration was necessary. In many experiments glass dishes with ground edge and fitted cover with ground groove were used. The pieces

were placed in these dishes in the solution and a number of the dishes were placed in larger glass jars. A liter or more of the same solution was then poured over the dishes to seal them and was allowed to remain in the large jar, which was then covered with a glass plate sealed on with vaseline and weighted. Here also all fluids were renewed at least every forty-eight hours. Later still I found that the worms or pieces would live in water in corked liter Erlenmeyer flasks with only a small bubble of air at the top. When the water in the flasks was changed every two to four days and not more than twenty-five or thirty large worms or their equivalent in smaller worms or pieces were introduced the worms could be kept indefinitely under these conditions without any injurious effects and showed as high a rate of metabolism as the animals kept in open dishes. In my later experiments the flasks have been used almost exclusively and have given very satisfactory results. Their only disadvantage is the necessity of removing the worms to other dishes for microscopic examination, but this is not serious if Erlenmeyer flasks are used for the animals can be dislodged with no great difficulty and without injury by means of a current from a large rubber bulb pipette. In consequence of the very small surface of the fluid exposed in the neck of the flask the loss of the anesthetic is very slight, even if the cork is not perfectly tight-fitting. Moreover, except in very low concentrations or after acclimatization to the anesthetic, the animals are usually at the bottom of the flask. It is certain therefore that decrease in concentration as a source of error is eliminated.

a. Alcohol. Series 71: August 10, 1905. Thirty worms 8 to 10 mm. in length and well fed were cut into two pieces, *a* including the region between the levels 1 and 5 in figure 1, and *b*, the region from 5 to 8, the posterior end in figure 1. These pieces were placed in 1.5 per cent alcohol at a temperature of about 22°C. A control series in water was not made in this case since everyone who has worked with *Planaria* knows that pieces like *a* and *b* give under normal conditions practically 100 per cent of normal animals.

After ten days in alcohol with a renewal of the mixture every forty-eight hours the pieces were examined. As far as they con-

cern the character of the head the results are given in table 1 in percentages. The different types of head distinguished in the table are described fully in earlier papers (Child, '11 c, '11 d). The normal head possesses equal, symmetrically placed eyes and the cephalic lobes are lateral to the eyes. In the teratophthalmic head the eyes are abnormal in position, number or form, in the teratomorphic head the anterior region fails more or less completely to develop and the cephalic lobes appear on the truncated front end of the head or may be fused together in the median line. The anophthalmic form shows a distinct anterior outgrowth but no eyes and finally in the headless form the anterior new tissue merely fills in the contracted cut surface.

TABLE I

	NUMBER OF PIECES	NORMAL	TERATOPH- THALMIC	TERATO- MORPHIC	ANOPH- THALMIC	HEADLESS	NEW PHARYNX	DEAD
a....	30	60	36.7					3.3
b....	30	16.7	26.7	13.3		3.3	40	40

These two sets of pieces *a* and *b*, which in water give results practically identical except for the position of the pharynx, show in alcohol a very great difference in regulatory capacity. The *a*-pieces show a lower mortality and form heads more frequently in alcohol than the *b*-pieces.

It is evident then that the regulatory processes in the two sets of pieces which under the usual conditions lead to practically identical results are not the same in their reaction to alcohol: at the level of the old pharynx head-formation is much less frequent than at a level near the old head. If we compare other levels between these, as I have done in other series, we find that under the same conditions their capacity for head formation lies between these two extremes.

These data demonstrate the existence of differences of some sort at different levels along the axis, but they give no certain information concerning their nature: that problem will be considered later.

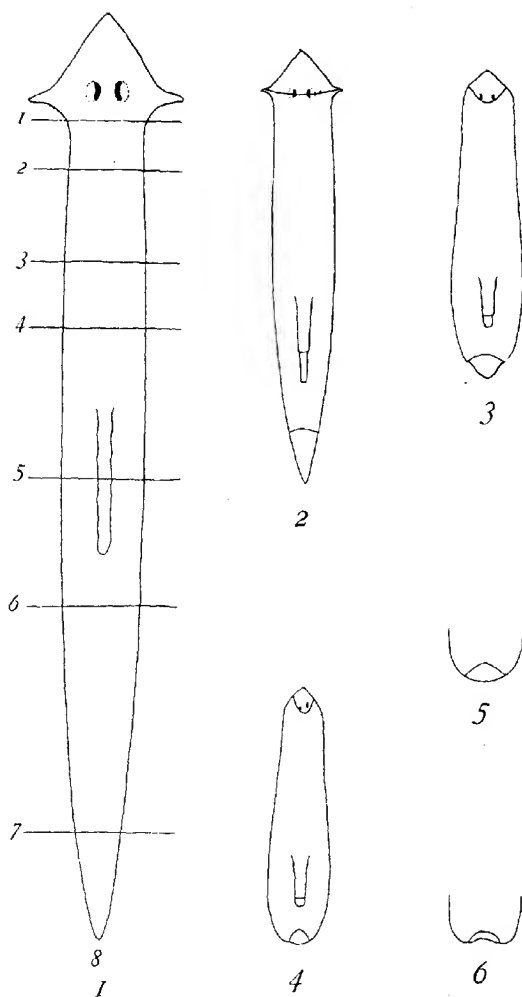
On the other hand, the examination of individual pieces indicates the existence of differences of some sort at different levels of each piece. In water under the usual conditions pieces like *a* resemble figure 2 after eight to ten days of regulation. A large new head is present, a considerable outgrowth of new tissue has appeared at the posterior end and the pharynx has regenerated a new posterior portion and has apparently migrated in the anterior direction in the body.

The appearance of the \bar{a} -pieces of series 71 after ten days in alcohol 1.5 per cent is indicated in figures 3 and 4, which are typical examples. Figure 3 shows the maximum posterior regeneration in the whole set of thirty pieces: in figure 4 the posterior regeneration is less and figures 5 and 6 show still other types of posterior ends. In two pieces only did an outgrowth of new tissue like that in figure 3 occur. The other pieces were all like figures 4, 5 and 6.

The pharynx shows much less regeneration in the alcohol pieces than in water, but its apparent migration in the anteriopdirection has not been wholly inhibited. As a matter of fact, however, the apparent migration of the pharynx in these pieces is merely the result of a shortening of the pharynx. The distance from the anterior end of the pharynx to the posterior end of the old tissue in figures 3 and 4 is no greater than the length of the anterior half of the pharynx originally present in the piece (fig. 1). In water where the apparent migration is more rapid and of greater extent (fig. 2) growth does undoubtedly occur in the region which thus comes to lie posterior to the pharynx.

The difference in shape in water and alcohol is also striking. In water (fig. 2) the posterior end is slender and tapering and the head region is as broad as any part of the body. In alcohol (figs. 3 to 6) the head is much smaller, and the posterior end is blunt and wider than any other part of the body.

It is evident then that in alcohol the regulatory processes at the posterior ends of the pieces are much more completely inhibited than those at the anterior ends. For the whole series the results are as follows: of the thirty *a*-pieces twenty-nine, 96.7 per cent, form heads and eighteen pieces or 60 per cent form normal heads,



Figs. 1 to 6 Effect of alcohol on regulation. Figure 1, diagrammatic, indicating levels of section. Figure 2, piece including region between levels 1 and 5 in figure 1: regulation in water. Figures 3 and 4, similar pieces: regulation in alcohol 1.5 per cent. Figures 5 and 6, posterior ends of alcohol pieces.

but in only two pieces (fig. 3) does anything resembling a normal posterior end appear and in these two cases the new outgrowth is still embryonic in character and contains no intestinal branches, while in water the intestinal branches enter the new tissue several days earlier. In all the other pieces the posterior new tissue merely fills the concave cut end and does not grow out beyond the contour of adjoining parts (figs. 4 and 5) and in some cases does little more than close the wound (fig. 6).

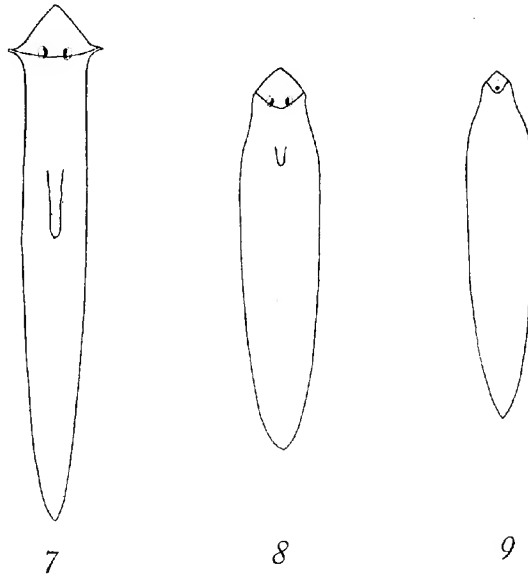
If such pieces are returned to water the posterior end gradually elongates and becomes more slender, though the new tissue shows no marked further increase, and the whole gradually approaches the characteristic shape of such pieces. If, on the other hand, the pieces remain in alcohol of the same concentration, 1.5 per cent, they show some degree of adjustment or acclimatization to the new environment and regulation may proceed somewhat farther than the stages figured above, but is always retarded or inhibited to a greater extent in the posterior than in the anterior region.

In the *b*-pieces conditions are somewhat different. These pieces (5 to 8, fig. 1) do not contain the old pharynx and though the posterior part of the old pharyngeal pouch is present it degenerates and the new pharynx forms independently of it. Regulation in these pieces consists essentially in the formation of a new head, a prepharyngeal and a pharyngeal region.

In water regulation occurs as indicated in figure 7: the new head is large and almost always normal and the new prepharyngeal region represents about the anterior third of the piece. In 1.5 per cent alcohol the head is much smaller (figs. 8 and 9), is often abnormal and there is less new tissue (fig. 9); the new prepharyngeal region is always shorter than in water (fig. 8), the pharynx is always small and in the more extreme cases may be entirely absent (fig. 9). That portion of the piece which redifferentiates into the prepharyngeal region always becomes narrower than more posterior regions of the piece (fig. 8) and even in cases where the pharynx is entirely absent a short region posterior to the new head is usually more or less narrowed (fig. 9). This narrowed region is the region where regulation has occurred in

such cases: posterior to it practically no changes have occurred in the piece.

In the above table only 56.7 per cent, i.e., seventeen of the pieces formed heads at all: all the others died except one piece which remained headless. A new pharynx appeared in 40 per cent of the whole number, i. e., in twelve pieces. It is clear that



Figs. 7 to 9 Alcohol. Figure 7, piece including region between levels 5 and 8 in figure 1: regulation in water. Figures 8 and 9, similar pieces: regulation in alcohol 1.5 per cent.

the process of pharynx-formation is affected to a much greater degree by the alcohol than that of head-formation for in five pieces, 16.7 per cent, heads but no pharynges appeared and in those cases where the pharynx did form it was always very small and remained so. Figure 8 represents the maximum regulation in the set after ten days in alcohol: the head is normal and though smaller than in similar pieces in water (fig. 7) is not extremely small, but

the pharynx is minute and never attains anything like the usual size in water. Figure 9 shows one of the more extreme cases in which the head is smaller and teratophthalmic and the pharynx is entirely absent. In all cases pharynx-formation is inhibited before head-formation; the latter can go on to a greater or less extent under conditions which completely inhibit the former.

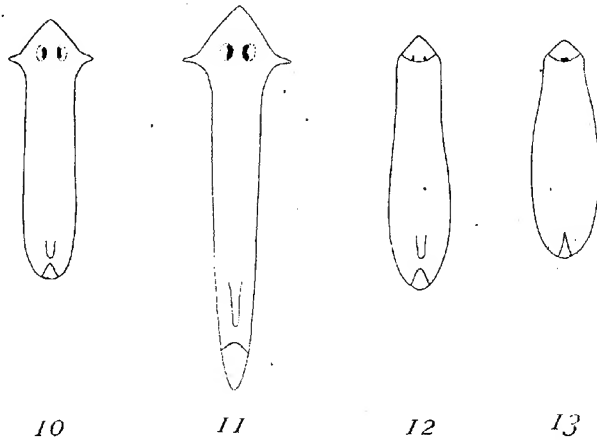
This series does not afford a direct comparison between the pharynx and the posterior end because in the *a*-pieces the old pharynx and in the *b*-pieces the old posterior end is present. It does show, however, that in the *a*-pieces head-formation may occur under conditions which inhibit the formation of a posterior end and in the *b*-pieces under conditions which inhibit pharynx-formation. Moreover, we see that head-formation is affected to a greater extent in the *b*- than in the *a*-pieces by a given concentration of alcohol. All of these facts indicate the existence of differences of some sort, apparently quantitative, in the dynamic processes at different levels along the axis, both in the original animal and in the piece.

Series 84 and 85. October 12, 1908. Well fed worms 12 to 14 mm. in length were used. Series 85 consists of twenty pieces including the old head and that portion of the body anterior to the level 4 in figure 1; these were kept in 1.5 per cent alcohol. Series 84 is a control of twenty similar pieces in water. Figure 10 shows the condition of the pieces of series 85 after eight days in alcohol, figure 11 the condition of the control in water after the same length of time. In figure 10 regulation posterior to the new pharynx is almost completely inhibited; no tail is formed and the postpharyngeal region does not elongate as in the control. The pharynx itself is of small size, but its formation is not inhibited. Evidently postpharyngeal regulation and tail-formation are more completely inhibited in this series than pharynx-formation.

Series 81. In this series the effect of placing the whole worms in alcohol for different lengths of time before operation was determined. On October 1, 1908, one hundred well fed worms 15 mm. in length were placed in alcohol 1.5 per cent in crystallizing dishes holding about $1\frac{1}{2}$ liters: these were filled almost full of the

fluid and covered with an accurately fitting glass plate, and the fluid was renewed daily. At intervals of two days ten pieces including the region between the levels 1 and 4 in figure 1 were cut and allowed to regulate in 1.5 per cent alcohol. In the first of these sets the worms had been in alcohol two days before the operation, in the second set four days, etc.

In general the series shows that the effect of the alcohol on regulation increases with the length of time in alcohol before section,



Figs. 10 to 13 Alcohol. Figure 10, piece including region anterior to level 4 in figure 1: regulation in alcohol 1.5 per cent. Figure 11, similar piece: regulation in water. Figure 12, piece including region between levels 1 and 4 in figure 1: from worm in alcohol 1.5 per cent two days before section: regulation in alcohol 1.5 per cent. Figure 13, similar piece from worm in alcohol four days before section.

the length of time after section being the same in all cases. Fig. 12 shows the characteristic condition of the pieces cut after the worms had been two days in 1.5 per cent alcohol and allowed to undergo regulation during fourteen days in alcohol of the same concentration. Five pieces remained alive at the end of this time and all were much alike. Tail-formation is almost completely inhibited but the pharynx is well developed though small.

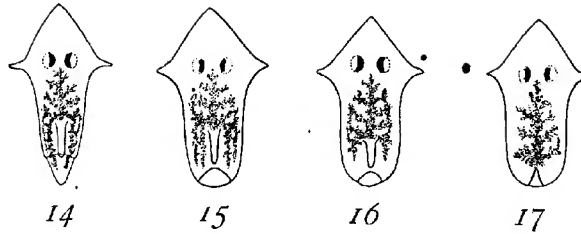
The head is usually normal as in figure 12, though occasionally teratophthalmic. In these five pieces no appreciable changes beyond the condition shown in figure 12 occurred before death.

Figure 13 shows a piece cut after the worms had been four days in alcohol and left in alcohol fourteen days after cutting. At the end of this time four pieces were alive. As indicated in the figure, regulation at the posterior end is limited to filling in the contracted cut surface and the pharynx is also entirely absent. Nevertheless these pieces form a head, though it is usually teratophthalmic. Here again the formation of a head occurs under conditions which inhibit the formation of pharynx and tail. No further regulation occurred in these pieces and they finally died in the alcohol.

In general the same axial relations appear in this series as in the preceding: the regulatory processes at the anterior end of the piece are less retarded or less often inhibited than those concerned with pharynx-formation and these in turn less than the processes of tail formation.

Series 69 and 70. In these series the effects of different concentrations of alcohol were compared. On August 4, 1908, pieces including the old head and the region anterior to the level 2 in figure 1 were cut from worms about 10 mm. in length. Of these pieces five were placed in water as control, five in 0.5 per cent alcohol, five in 1 per cent and five in 2 per cent alcohol. The room temperature at the time of the experiment was rather high during the day, often reaching 26°C. and regulation occurred rapidly. At the end of five days the pieces in water were approximately like figure 14. New tissue had grown out at the posteriorly completed; the postpharyngeal intestinal branches already extended well into the new tissue. Figure 15 shows the usual condition after five days in 0.5 per cent alcohol. The formation of the pharynx and of the two lateral intestinal branches has taken place, but the posterior new tissue does not form a functional tail and the intestinal branches have not grown into it.

Figure 16, from 1 per cent alcohol after five days, shows still further decrease in the posterior new tissue. No tail is present



Figs. 14 to 17 Effect of different concentrations of alcohol: pieces include region anterior to level *l* in figure 1. Figure 14, water. Figure 15, 0.5 per cent alcohol. Figure 16, 1 per cent alcohol. Figure 17, 2 per cent alcohol.

but the pharynx has developed and intestinal regulation in the pharyngeal region has begun.

In figure 17 a piece after five days in 2 per cent alcohol is shown. All regulation except closure of the wound is inhibited. This is the only one of the five pieces that lived five days.

These series show that the formation of a pharynx and of the lateral intestinal branches in the pharyngeal region may occur under conditions which completely inhibit the formation of a structurally differentiated, functional tail.

The records of numerous other alcohol series might be given, but all are of essentially the same character. Everywhere the process of tail-formation is inhibited by less extreme conditions than those necessary for inhibition of the processes characteristic of more anterior levels. The process of pharynx-formation shows a greater resistance to alcohol than that of tail-formation, but less than that of head-formation. In pieces from different levels, on the other hand, the same regulatory process is differently affected by the same concentration of alcohol. All the results obtained along this line with alcohol point to the existence of a dynamic axial gradient in the original worm and in the piece.

b. Ether. The results obtained with 0.4 to 0.5 per cent ether are in general similar to those described above for alcohol. The same differences appear along the axis in the effect of the ether upon the regulatory processes. Ether inhibits the outgrowth of new tissue to a somewhat greater extent than alcohol so that

regulation in ether becomes almost entirely a process of redifferentiation as contrasted with regeneration in the stricter sense. The data of one series are given.

Series 92. November 4, 1908. Well fed worms 15 to 17 mm. in length were used. The series consists of ten prepharyngeal pieces comprising the region between the levels 1 and 2 in figure 18, and ten postpharyngeal pieces (3 and 4, fig. 18). So far as the results can be tabulated they are given in percentages in table 2. Since each lot consists of ten pieces the percentage figures are ten times the number of pieces. The *a*-pieces in the table are the prepharyngeal, the *b*-pieces the postpharyngeal pieces.

TABLE 2

NUMBER OF DAYS IN ETHER	PIECES	ALIVE	NORMAL HEAD	TERATOPI- THALMIC	EYES NOT YET VISIBLE	PHARYNX PRESENT	ANTERIOR DISINTE- GRATION
12	<i>a</i>	100	10	10	50	30+	30
	<i>b</i>	100	0	0	100	0	0
18	<i>a</i>	30	20	10	0	30	0
	<i>b</i>	30	20	0	0	0	10

The table shows the differences in the pieces after twelve and after eighteen days in ether. After twelve days one (10 per cent) of the prepharyngeal pieces has a normal head, one a teratophthalmic head and at least three pieces (30 per cent +) show a new pharynx. Three other pieces are disintegrating at the anterior end instead of undergoing regulation and will soon die. The postpharyngeal pieces at this stage show no heads and no pharynges.

After eighteen days in ether only three pieces are alive in each set. Two of the three prepharyngeal pieces are normal and one teratophthalmic and all have pharynges, while of the postpharyngeal pieces two are normal as regards the head, but are without pharynges and one is degenerating anteriorly. It is evident that regulation is less completely inhibited in the prepharyngeal than in the postpharyngeal pieces. Heads and eyes appear earlier and pharynges appear only in the prepharyngeal pieces.

A few figures will serve to supplement the tabulated data. Figure 19 shows the prepharyngeal piece with normal eyes after

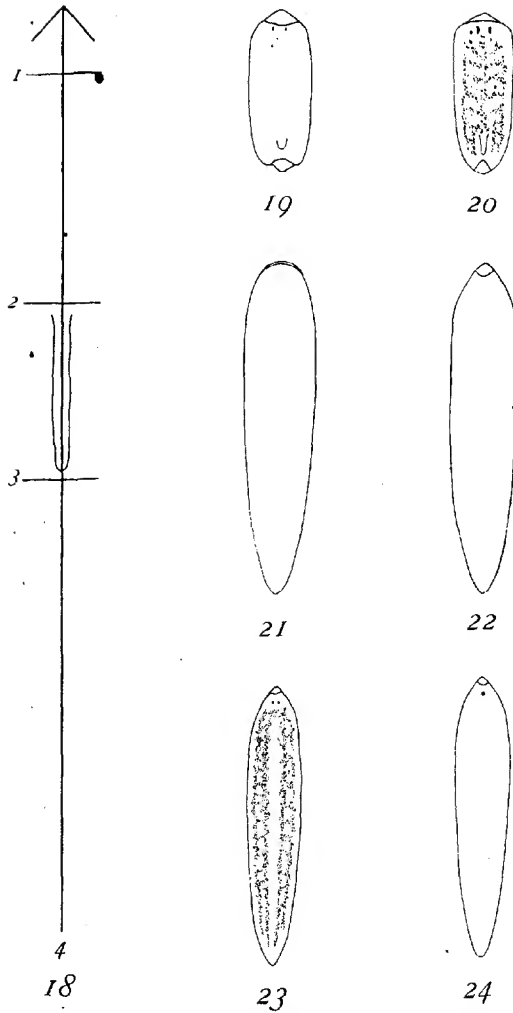
twelve days and figure 20 another piece after eighteen days. Neither of these pieces has formed what can be called a tail but both show a small pharynx and in both a normal head has formed, chiefly by a process of redifferentiation. Intestinal regulation has occurred only so far as the formation of the pharynx has displaced and obliterated parts of the original intestine.

Figures 21 and 22 show two of the postpharyngeal pieces after twelve days. In figure 21 a condition often seen in ether is shown: here the cut surface at the anterior end has not contracted in the usual manner but has remained widely open and a very thin layer of new tissue covers it. In figure 22 the anterior end contracted and a small amount of new tissue arose. These two figures represent the extremes of difference in the pieces at this stage. None show either eyes or pharynx.

Figure 23 shows one of the two pieces which lived long enough to develop eyes. The head, so far as it has developed, is almost wholly the result of redifferentiation and shows no trace of auricles, and the piece contains no pharynx. Such pieces examined under pressure show almost no trace of intestinal regulation; the lateral intestinal branches usually meet anteriorly, but no prepharyngeal axial intestine is formed (fig. 23). Regulation is evidently confined entirely to the head region and even there is limited chiefly to the nervous system with which the appearance of the eyes is closely connected.

This series shows particularly well how certain morphogenetic processes can be eliminated while others still continue under the same external conditions.

Figure 24 shows a postpharyngeal piece from another ether series: here a still more extreme modification of regulation appears. The only externally visible evidences of regulatory morphogenesis are the minute area of new tissue at the anterior end and the single median optic pigment spot. Examined under pressure, such pieces show small cephalic ganglia more or less fused (Child and McKie, '11). No trace of a pharynx exists and the alimentary tract is either similar to that in figure 23 or the two parts may be entirely separate anteriorly.



Figs. 18 to 24 Effect of ether on regulation. Figure 18, diagrammatic, indicating levels of section. Figures 19 and 20, pieces including the region between levels 1 and 2 in figure 18: regulation in 0.4 per cent ether. Figures 21 and 22, pieces including region between levels 3 and 4 in figure 18: twelve days in ether. Figures 23 and 24 similar pieces after eighteen days in ether.

With chloretone results of the same general character as those described above for alcohol and ether have been obtained.

2. *Experiments with potassium cyanide*

The cyanides I have found of great value in my attempts to analyze the processes of regulatory morphogenesis. The results, while of the same general character as those obtained with the anesthetics, can be more exactly controlled and the more extreme modifications and inhibitions are readily obtained without the high mortality which accompanies the use of the anesthetics.

In my experiments with KCN I have used corked Erlenmeyer flasks as described above and have often kept several hundred pieces, fifty in each flask, for two or three weeks in KCN, renewing the solution every forty-eight or ninety-six hours, without losing a single piece, yet the solution was sufficiently concentrated to alter the regulatory capacity of the pieces very greatly. In all such experiments where controls in water are necessary these are also kept in corked flasks, although I have not been able to discover that the results as regards regulation differ whether the pieces are kept in water in corked flasks or in shallow open dishes, provided, of course, that the water in the flasks is changed often enough.

Here only one of my cyanide series need be given since all points essential to the present purpose are illustrated by it and the results of other series are in general similar.

Series 409. June 24, 1911. Well fed worms 16 to 20 mm. were used: from these pieces were cut as follows:

- 1a 25 pieces including the region 1-3 in figure 25
- 1b 25 pieces including the region 3-5 in figure 25
- 2a 25 pieces including the region 1-2 in figure 25
- 2b 25 pieces including the region 2-3 in figure 25
- 2c 25 pieces including the region 3-4 in figure 25

These pieces were placed in KCN 1.0×10^{-3} m in liter flasks, the solution being renewed every four days. Eight days after the beginning of the experiment a rise of several degrees in the temperature of the room occurred and the pieces began to die. Ten days after

the pieces were placed in KCN all were examined and since the high temperature made it probable that all would die in a few days if left in the KCN, all that were then alive were returned to water. The condition of the pieces at the end of the ten days in KCN was as follows:

Nine *1a*-pieces were alive and were all of the type shown in figure 26. Normal heads were present in all, but tail-formation was completely inhibited: the new tissue merely closed the wound and did not even fill the concavity of the contracted cut surface. The anterior half of the old pharynx, which the piece contained from the beginning, was much reduced and showed no signs of regeneration.

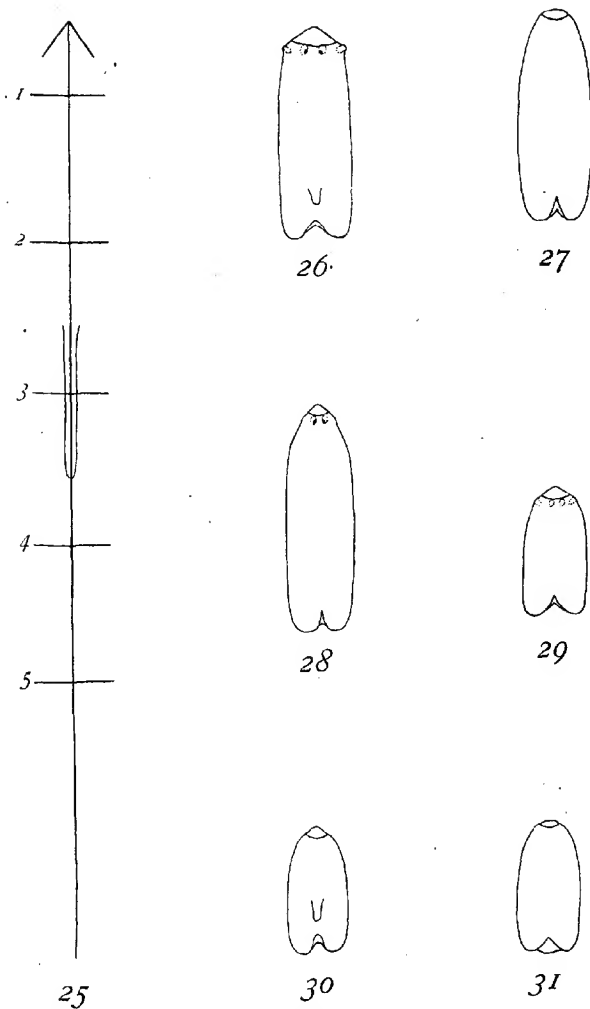
Of the *1b*-pieces seventeen were still alive: of these fifteen were like figure 27 and two like figure 28. None of them showed any trace of pharynx-formation.

The condition of the shorter pieces, *2a*, *2b*, *2c* after ten days in KCN is shown in figures 29 to 31. The *2a*-pieces, of which twenty-one were alive, were all much alike (fig. 29). An outgrowth of new tissue had appeared at the anterior end and eyes and auricles were distinguishable by their unpigmented areas, but no pharynx had formed and at the posterior end nothing beyond closure of the wound had occurred.

Of the *2b*-pieces seventeen were still alive, all similar to figure 30. The anterior new tissue in these pieces is less in amount than in *2a*, neither eyes nor auricles have appeared as yet, the anterior half of the pharynx, which these pieces contain, shows no regeneration and regulation at the posterior end is limited to wound-closure.

Seventeen of the *2c*-pieces were alive: the anterior outgrowth was less than in *2b* and neither pharynx nor posterior end had developed. Figure 31 shows the character of these pieces.

The comparison of these five sets is of interest. In every piece the regulatory processes at the posterior end are more completely inhibited than those at the anterior end, moreover, the degree to which the formation of the head is affected by the KCN varies with the level of the pieces in the original body. Head-formation is affected less in the *1a*-pieces (fig. 26) than in the more posterior



Figs. 25 to 31 Effect of KCN on regulation. Figure 25, diagrammatic, indicating levels of section. Figure 26, piece including region between levels 1 and 3 in figure 25: after ten days in KCN $\overline{1000000}$ m. Figures 27 and 28, pieces including regions between levels 3 and 5 in figure 25: after ten days in KCN $\overline{1000000}$ m. Figure 29, from region 1 2 in figure 25. Figure 30, from region 2 3. Figure 31, from region 3 4: all ten days in KCN $\overline{1000000}$ m.

1b-pieces (figs. 27 and 28) and anterior regulation in the *2a*-pieces (fig. 29) is less affected than in the *2b*-pieces (fig. 30) and in these less than in the *2c*-pieces (fig. 31). In short the same gradient appears in these experiments as in those with alcohol. If, as has been suggested, KCN retards the oxidation processes the results of the experiments suggest the existence of a gradient in the rate of the oxidation processes along the axis. As will appear later there is very strong evidence along other lines for the existence of such a gradient.

Incidentally two other points of interest concerning these pieces may be noted: first, in the KCN the development of the optic pigment cups is more completely inhibited than that of the unpigmented optic areas. In the *1a*-pieces the optic pigment cups are represented by small dots, while the unpigmented areas are much more nearly the usual size; the same condition exists in those *1b*-pieces which develop eyes (fig. 28). In the *2a*-pieces this differential effect is still more striking for here the pigment cups are absent, though the unpigmented areas are well marked (fig. 29).

Second, the differentiation of the sensory unpigmented areas of the auricles is less completely inhibited than the outgrowth of the auricles. In the *1a*-pieces (fig. 26) there is a barely appreciable outgrowth, but the sensory areas are well developed and in the *2a*-pieces (fig. 29) the sensory areas appear without any trace of outgrowth. These results show some of the possibilities of analysis of regulatory morphogenesis.

3. *Experiments with temperature*

Series 310, 311, 314. January 9, 1911. These series show the effect of different temperatures upon the regulatory processes at the posterior ends of short anterior pieces. The pieces used included the head and the short portion of the body anterior to the level 1 in figure 32; all were cut from well-fed animals 16 to 18 mm. in length from the same stock. Pieces of this size and character were chosen because they represent in a sense a critical length for this region of the body and for medium temperatures, i.e., at temperatures of 18° to 22°C. such pieces sometimes give rise to

normal animals and sometimes fail entirely to develop a pharynx and posterior end (tailless forms). Table 3 gives in percentages the results of regulation of such pieces at three different temperatures:

TABLE 3

SERIES	TEMPERATURE	NUMBER OF PIECES	TAILLESS	NORMAL	DEAD
314 I.....	26° to 28°	25	0	88	12
310.....	20°	50	22	70	8
311 II.....	10°	50	44	54	2
314 II.....					

Each of the three series combined in the table was originally intended for comparison of the effects of different temperatures, but in series 310 and 311 the high temperature lots died because too high a temperature was used: these two series are therefore incomplete but their combination with series 314 gives comparative results for three temperatures.

The percentages of 'tailless' and 'normal' in the table represent final results, i.e., the tailless forms were not cases in which formation of the posterior end was retarded. Very different lengths of time were necessary at the different temperatures; at the highest temperature regulation was complete in about four days, at 20° in eight to nine days and at 10° nearly a month was necessary. The pieces were kept considerably longer than this in order to make sure that tails did not appear later, but as a matter of fact the tailless animal can be readily distinguished from one which is going to form a tail after a relatively early stage by the thickening of the posterior region which appears in the tailless forms.

The table shows clearly that the percentage of tailless forms increases as the temperature falls. The lower the temperature at which regulation occurs the less frequently do the pieces become wholes. This variation of the regulatory capacity with temperature is apparently due to the fact that at lower temperatures the rate of reaction at the posterior end of the piece is more frequently below the level necessary for tail-formation than at higher. So far the results have no relation to the axial gradient, but when we examine and compare the pieces which have undergone regulation

at different temperatures certain points concerning the gradient appear.

Figure 33 shows the characteristic result after eight days at a temperature of 26° to 28° . The posterior outgrowth of new tissue is long, the new intestinal branches have already extended to its posterior end, the pharynx is advanced in development and the change in proportion is marked. The pieces have decreased considerably in size because of their high rate of metabolism and the absence of food. All the high temperature pieces are essentially like this.

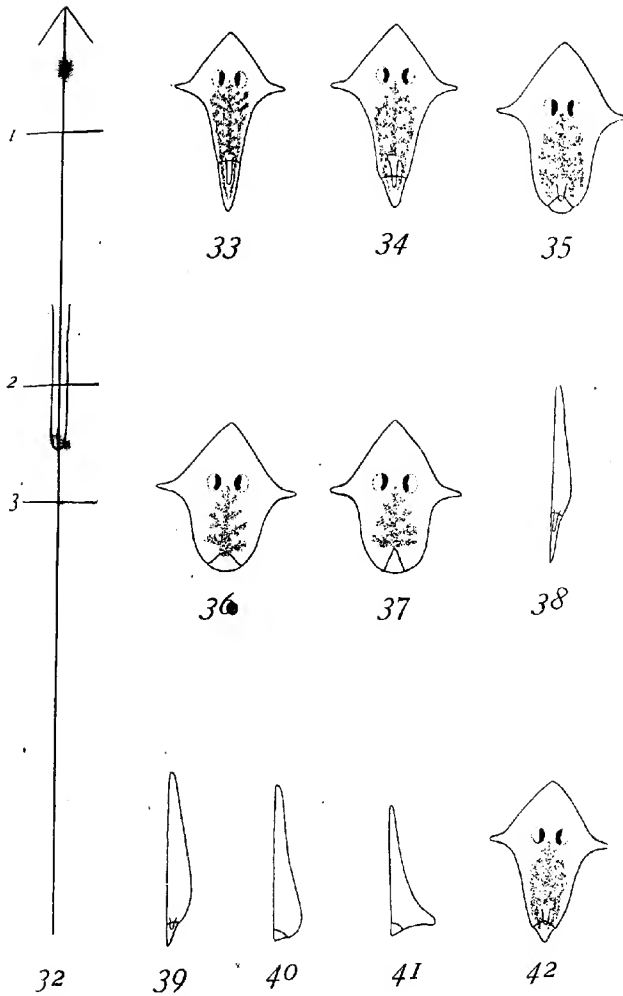
Figure 34 represents a normal whole after eleven days at a temperature of 20° . The outgrowth of new tissue is much shorter than in figure 33 and the posterior intestinal branches have not developed as far into the new posterior end, but the pharynx is about the same size in the two cases. In other words the extreme posterior region of the body is less highly differentiated in figure 34 than in figure 33: its development has been retarded to a greater extent, or has ceased at a slightly earlier stage, while farther anteriorly, i.e., in the pharyngeal region, the development is much the same in the two cases.

In figure 35 a normal whole after thirty-six days at 10° is shown. The posterior new tissue is short and blunt and the posterior intestinal branches have not penetrated it at all: its development as a tail has ceased at a much earlier stage than in the preceding cases. In this case the development of the pharynx also ceases at a much earlier stage than at higher temperatures. It remains small and short and apparently does not attain any considerable degree of motility. Here, too, the change in proportion is practically absent because the locomotion of the piece is slow and the new tail scarcely functions at all as an organ of attachment (Child, '09). In fact, it is perhaps a question whether the posterior outgrowth in these pieces can be called a tail or not. Since it is impossible to determine just where it ceases to be a tail, I have called all cases wholes in which a pharynx is present and the formation of the posterior intestinal branches has begun. It is evident at any rate that in such pieces the morphogenetic processes in the postpharyngeal region are more completely inhibited or cease at an earlier stage than in the pharyngeal region.

At the highest temperature all of the pieces form wholes: at a temperature of $20^{\circ} 22$ per cent, and at $10^{\circ} 44$ per cent remain tailless. There is some difference in the amount of new tissue in the tailless forms: the usual type at 20° is shown in figure 36 and that at 10° in figure 37, though some animals like figure 36 occur at 10° and some like figure 37 at 20° . In all of these cases regulation at the posterior end is almost completely inhibited. The growth of new tissue does not proceed far beyond closure of the wound and the region is not used as a tail by the animal. The difference in this respect is striking. These tailless forms are quite unable to adhere to the substratum by their posterior ends and so are dislodged by very slight movements of the water, while the individuals with tails in the same dish hold tightly to the substratum like normal animals. In such pieces the formation of both the postpharyngeal and the pharyngeal region is completely inhibited.

The longitudinal optical section of the different pieces shows some points of interest. Figure 38 is the section of the high temperature wholes, figure 39 that of the low temperature wholes and figure 40 that of the tailless forms. In these last no posterior elongation occurs so long as they are kept under the same conditions, but in later stages the thickening in the posterior region of the body increases and often a peculiar dorsal hump or outgrowth (fig. 41) appears, which contains the continuation of the alimentary tract. A study of the living animals shows that these dorsal elevations arise in the region where the pressure of the intestinal contents is greatest when they are forced posteriorly and often a bulging of the body-wall is visible in this region whenever contraction occurs, even before the permanent outgrowth has formed. These dorsal humps are undoubtedly the result of the

Figures 32 to 42 Regulation at different temperatures: all pieces represent the region anterior to the level *l* in the diagrammatic figure 32. Figure 33, regulation at high temperature. Figure 34, regulation to 'whole' at medium temperature. Figure 35, regulation to 'whole' at low temperature. Figure 36, 'tailless' form: regulation at medium temperature. Figure 37, 'tailless' form: regulation at low temperature. Figures 38 to 41, longitudinal optical sections of whole and tailless forms. Figure 42, whole produced by subjecting a tailless form to higher temperature.



reaction of this region to the altered mechanical conditions resulting from the absence of the posterior end.

At 10° 44 per cent, twenty-two pieces, were tailless after thirty-six days. These tailless pieces were then brought to a temperature of 20° and left for eight days. At the end of this time ten of the pieces had developed tails pharynges and posterior intestinal branches as indicated in figure 42. The other twelve pieces showed no signs of tail-formation. This result affords a confirmation of the results given in table 3 for 20°. At that temperature 22 per cent, eleven pieces, remained tailless; at 10° 44 per cent, twenty-two pieces remained tailless, but when these tailless pieces were raised to 20° only 24 per cent of the total remain tailless. This percentage is almost the same as that of the pieces kept throughout at 20° (22 per cent).

Figure 42 shows the result of acceleration of the regulatory processes, while the preceding figures show the results of retardation. It is evident from figure 42 that morphogenetic development has occurred to a greater extent at the level of the pharynx than in the postpharyngeal region; in other words, when the rate of reaction is accelerated during development the morphological effect is greater or appears more rapidly in the more anterior region.

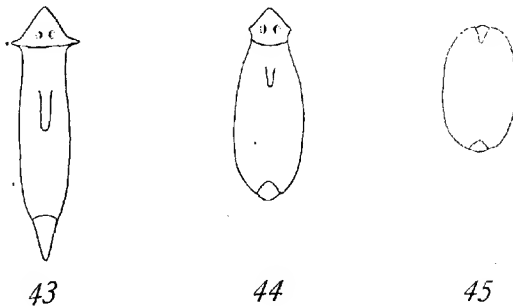
In these series we are concerned only with the pharyngeal and postpharyngeal regions but evidence of the existence of a dynamic gradient appears in these regions in the different effects on morphogenesis at different levels of retardation and acceleration of the dynamic processes.

Series 291. December 21, 1910. From well fed worms 16 to 18 mm. in length pieces including the region between the levels 2 and 3 in figure 32 were cut. Fifty of these pieces underwent regulation at a temperature of 8° to 10°C. and fifty at 28° to 30°C. The final results, tabulated in percentages are as follows:

TABLE 4

NUMBER OF PIECES	TEMPERATURE	NORMAL	TERATOPH- THALMIC	TERATO- MORPHIC	ANOPH- THALMIC	HEADLESS	DEAD
50	28° to 30°	74	20	2	2	0	2
50	8° to 10°	26	22	20	10	22	0

The table shows very great differences in the character and frequency of head-formation at the different temperatures, but the evidences for the existence of an axial gradient appear only on examination of the individual pieces. At the higher temperature 74 per cent of the pieces were like figure 43, with large head, well developed pharynx and long, pointed posterior outgrowth. The alimentary tract (not shown in the figure) is well developed in the new posterior end. Figures 44 and 45 represent the two extremes after thirty-six days at the lower temperature. Only 26 per cent of the pieces were like figure 44, the others being intermediate between it and figure 45, or like the latter. In all of these pieces



Figs. 43 to 45 Regulation at different temperatures: pieces include region between levels 2 and 3 in figure 32. Figure 43, regulation at high temperature. Figures 44 and 45, regulation low temperature.

at low temperature the outgrowth at the posterior end of the piece is almost entirely inhibited. Even in figure 44, which represents the most complete regulation at the lower temperature, the new tissue merely fills the contracted wound and shows no actual outgrowth beyond the contour of adjoining parts. There is no trace of posterior intestinal regulation but a normal though small pharynx and a normal head have developed. Evidently the low temperature inhibits posterior regulation to a very large extent, but 68 per cent of the pieces in which posterior regulation was like that in figures 44 and 45 developed heads of some kind and 26 per cent developed normal heads. In the extreme headless

type at low temperature (fig. 45) practically all regulation beyond wound-closure has been inhibited. The important feature of the series for present purposes is the development of heads at a temperature which practically inhibits the development of the posterior end.

In general the temperature experiments give results similar to those obtained with anesthetics and KCN. They indicate the existence of an antero-posterior dynamic gradient. Head-formation and the development of a small pharynx may occur at a temperature which practically inhibits development of the posterior end. The low temperature affects pharynx-formation more than head-formation and the development of the posterior end more than either.

The results with different temperatures are less extreme than those obtained with KCN and the anesthetics, but they are of the same character and since we know that the changes in organisms produced by different temperatures are primarily quantitative, they serve to confirm the conclusion that the effects of the other external agents are likewise primarily quantitative.

4. Other experimental conditions

With various other experimental conditions the results are essentially similar to those already described. In pieces from worms in extreme stages of starvation regulation at the posterior end is retarded to a greater extent than at the anterior end, the basis of comparison being of course well fed worms. Absence of food undoubtedly results sooner or later in a decreased rate of metabolism (Child, '11 b) and in pieces with this decreased rate the regulatory morphogenetic development at the posterior end is retarded or inhibited to a greater extent than at the anterior end.

Similarly the presence of metabolic products of *Planaria* in the water undoubtedly decreases the rate of metabolism and the effect on regulatory morphogenesis is similar to that of starvation or low temperature, though it may be greater and in extreme cases approaches that obtained with the anesthetics. In the second paper of this series some of the results obtained with metabolic products in the water were described in another connection (Child,

'11 e, pp. 203-204) and it was pointed out that the formation of a head, though often a teratomorphic head, was still possible in pieces where the development of the pharynx was completely inhibited.

There can be no doubt that a variety of other external agents which decrease metabolism will produce similar results, but since the present investigation is primarily concerned with the internal processes and conditions rather than with the external factors which produce them, no attempt has been made thus far to extend the experimentation to a great variety of substances. It has seemed preferable to acquire a more extended knowledge of the action of a few agents and conditions as a basis for further work. Sooner or later of course it will be desirable to compare the effects of a great variety of agents and such comparison will undoubtedly bring to light facts of much interest, but for work of this character an adequate basis is absolutely essential.

C. THE AXIAL FACTOR IN RELATION TO ANTERIOR AND POSTERIOR ZOIDS

In the experiments described above only the axial gradient within the limits of a single zooid has been considered. Within these limits the gradient is more or less uniform but in the passage from the posterior end of an anterior to the anterior end of a more posterior zooid it shows a marked change and in general the posterior zooids are in a somewhat different dynamic condition from the anterior zooid.

• Attention was called above (p. 105) to the fact that in the regulation of pieces under constant conditions the passage from the posterior region of the anterior zooid to the anterior region of the second zooid is clearly marked by a change in the regulatory capacity of the pieces. Pieces of given length from the anterior region of the second zooid show a much greater capacity to form normal heads and so to undergo complete regulation than do the pieces anterior to them in the posterior region of the first zooid: in other words, the pieces from the second zooid are physiologically more 'anterior,' more like pieces from the region near the head than those which lie just anterior to them in the body. It

was this difference which led to the recognition of the existence of posterior zooids (Child, '06, '09, '11 c, '11 f).

Under certain experimental conditions the axial factor in relation to the anterior and posterior zooids appears even more clearly than under the usual conditions. Here, as in the experiments described above, various conditions, e.g., different temperatures, alcohol and other anesthetics, KCN, etc., may be used to bring out these differences. Such experiments show beyond a doubt that the zooids are marked off more or less clearly by differences in dynamic conditions along the main axis. But the actual results as regards the regulation of the pieces differ very widely, in certain cases diametrically, according to the manner in which the reagent or condition is used in the experiment. At present, however, it is desired merely to show that the posterior zooids are distinguishable from the anterior by means of this dynamic axial factor. The problem involved in the different effects of a given agent under different conditions will be taken up later.

Only a few of my experimental series which bear upon this point are given below: KCN and temperature series are selected because they demonstrate the point clearly and have a much lower mortality than the series with anesthetics. In all cases the series are primarily concerned with the dynamics of regulation rather than with the point considered here which is merely an incidental result.

In order to avoid repetition it may be stated that in all the series given below worms 18 to 20 mm. in length were used in these worms the anterior zooid usually included about half the total length and the posterior half consists of either two or three well-defined zooids. In figure 46 the region in which the anterior end of the second zooid occurs is indicated by the shaded area at *xx*. The position of the boundary between the two zooids varies in different individuals and the length of the shaded area in the figure represents approximately the limits of variation, while the frequency of its occurrence at different levels within this limit is roughly indicated by the density of the shading.

Near the posterior end of the body in these worms a third zooid or a series of short zooids (Child, '11 f) exists and the region of the boundary between it and the second zooid or its descendants is

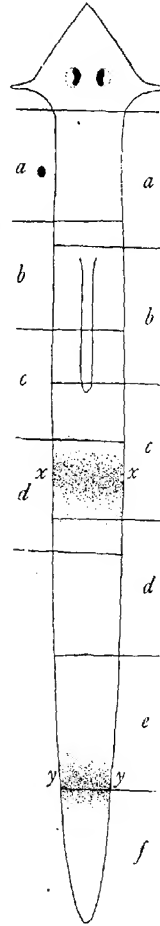


Fig. 46 Diagram, indicating levels of section. The two chief regions of fission are indicated by stippling at *xx* and *yy*; the length of stippled region indicates approximately the range of variation of the level of fission in different animals of the same size from the same stock and the density of shading indicates the frequency at different levels.

indicated in figure 46 by the shaded area, the length of the area and the density of shading representing as before approximately the limits of variation of position and the frequency. In many cases among these worms the second zoöid has undoubtedly divided into two, as the results of experiments indicate.

In all cases the worms had been fed three times a week with beef-liver for at least two months before the experiments began. When the worms are fed as often as this only a comparatively small part of the stock reacts at any one feeding, the others not being sufficiently hungry. I have found by experience that there is no object to be gained by feeding stocks oftener than this when they are kept at a temperature of about 20°C. Hence I have called this maximal feeding.

All worms used in the experiments, except where temperature was the experimental factor, were kept in the same room at a temperature ranging between 18° and 22°C. and in darkness. In all experiments the controls and the experimental lots were kept under conditions as nearly alike as possible, except for the one experimental factor.

1. Cyanide and temperature experiments

Series 432 I and II. November 28 to December 21, 1911. In this series the four pieces *a-d*, as indicated on the *left* side of figure 46 were used. It will be seen from figure 46 that the pieces *a*, *b* and *c* include practically the whole length of the first zoöid except the head, while the piece *d* consists in large part of the anterior region of the second zoöid. In consequence of the variation of the level of fission the posterior end of piece *c* sometimes includes a small part of the second zoöid, while at the other extreme *d* is mostly within the first zoöid. In lots of fifty pieces, however, most of the *c*-pieces are within the first zoöid and most of the *d*-pieces are largely within the second zoöid. If there is a difference in the dynamic conditions between the posterior region of the first and the anterior region of the second zoöid it should appear between pieces *c* and *d*.

Lots of fifty each of *a*, *b*, *c*, *d* were placed in water at a temperature of 18° to 22°C. as a control and similar lots of fifty each were

cut in water and placed in KCN $\frac{1}{1000}$ m. at the same temperature as soon as the cutting of the series was completed. Each lot of both the control and the KCN was kept in a liter Erlenmeyer flask filled with liquid except for a small air-space at the top, and corked. The KCN solution was renewed every two days and for the sake of uniformity the water of the controls was also changed as the same time. The KCN-pieces remained in the cyanide from November 28 until December 11 and were then rinsed and placed in water. All pieces were examined December 19 to 21. The results, so far as they concern the character of the anterior end, appear in table 5. *I* is the control in water, *II* the

TABLE 5

PIECES	NORMAL	TERATOPH- THALMIC	TERATO- MORPHIC	ANOPH- THALMIC	HEADLESS	DEAD
<i>Ia</i>	84	16	0	0	0	0
<i>IIa</i>	6	74	10	4	6	0
<i>Ib</i>	10	72	0	10	8	0
<i>IIb</i>	0	56	14	10	20	0
<i>Ic</i>	10	28	2	18	42	0
<i>IIc</i>	2	64	8	10	16	0
<i>Id</i>	34	58	2	4	2	0
<i>IIId</i>	10	82	0	2	2	4

pieces in cyanide. The results are given in percentages which are in all cases exactly double the actual number of pieces.

In the first place, the control pieces show the usual axial gradient described in the first paper of this series (Child, '11 c). In *Ia* 84 per cent are normal, 16 per cent teratophthalmic, a total of 100 per cent with eyes; in *Ib* only 10 per cent are normal and 72 per cent teratophthalmic, a total of 82 per cent with eyes; in *Ic* 10 per cent are normal, 28 per cent teratophthalmic and 2 per cent teratomorphic, a total of 40 per cent with eyes. The 10 per cent of normal heads in *Ic* represents those cases in which *Ic* contained a considerable portion of the second zoöid. Pieces of this length which are entirely from the posterior region of the first zoöid never produce normal heads under the conditions of this series.

The *Id*-pieces, on the other hand, which are usually largely within the second zoöid, show a marked increase in the capacity to produce eyes and normal heads. Here 34 per cent are normal, 58 per cent teratophthalmic and 2 per cent teratomorphic, a total of 94 per cent with eyes. The controls then show a decrease in the frequency of formation of eyes and heads in the posterior direction along the axis of the first zoöid, while at the anterior end of the second zoöid a marked increase in the frequency of eyes and heads appears.

Comparing the KCN-pieces with these, we see that in *IIa* only 6 per cent are normal as compared with 84 per cent in *Ia*, while 74 per cent are teratophthalmic and 10 per cent teratomorphic, as compared with 16 per cent in *Ia*. The remaining 10 per cent of *IIa* are anophthalmic or headless while none of *Ia* are of this character. Here then the effect of the KCN is a very great decrease in the regulatory capacity and particularly in the frequency of normal as compared with teratophthalmic heads.

In *IIb* no normal forms appear, 56 per cent are teratophthalmic and 14 per cent teratomorphic, a total of 70 per cent with eyes, the remaining 30 per cent being either anophthalmic or headless. This total, 70 per cent, is almost exactly the same as the total percentage of eyes, 72 per cent, in *Ib*, but in *IIb* 14 per cent of this total are teratomorphic, moreover, *IIb* shows 20 per cent of headless forms, as compared with 8 per cent in *Ib*. In short, the differences between the control and the KCN-lot in the *b*-pieces are nowhere very great: any one of the differences alone would be within the limits of error, which, as I have determined by comparing parallel series, are from 10 per cent to 20 per cent, being higher in the posterior than in the anterior pieces. Taken together, however, the figures in the different columns show beyond a doubt that the KCN decreases the regulatory capacity of the *b*-pieces, but they also show, when compared with the *a*-pieces, that the decrease is much less than in those. Apparently the KCN has less depressing effect on the regulation of the *b*-pieces than on that of the *a*-pieces.

In *IIc* 2 per cent normal appear as compared with 10 per cent in *Ic*: this difference by itself is not great enough to be regarded

as important. But 64 per cent of *IIC* are teratophthalmic, as against 28 per cent in *Ic* and 8 per cent teratomorphic as against 2 per cent in *Ic*. The total with eyes for *IIC* is 74 per cent, for *Ic* only 40 per cent. On the other hand, only 26 per cent of *IIC* are anophthalmic and headless, as against 60 per cent in *Ic*.

The effect of the KCN on the *c*-pieces is a very evident increase in the regulatory capacity of the pieces. At first glance such a result seems paradoxical, but as a matter of fact it is characteristic, not only for KCN but for the anesthetics, when used in a certain way. A full discussion of the reasons for this result in the *c*-pieces is postponed to a later paper. At present it need only be said that if the process of head-formation in these pieces were the restitution of a missing part, i.e., if it were in direct correlation with the processes in the other regions of the piece, such a result would be impossible. If, on the other hand, the formation of a new head in a headless piece represents the formation of a new individual head first and a process which occurs in spite of the other parts of the piece, then it becomes easy to understand how under certain conditions a depression of the piece should increase the frequency of head-formation. The formation of the new head in a headless piece of *Planaria* is a process of exactly this kind. The new head forms from cells which are involved in the wound-reaction and whether a given piece shall form a head or not depends on whether the region concerned in wound-reaction becomes sufficiently dominant over other parts to be self-determining and to be able to grow at their expense. If the required degree of dominance is attained a new head forms and reorganizes the piece in the antero-posterior direction. If the two regions remain more or less exactly balanced the piece remains headless or anophthalmic and finally, if the old part remains the dominant component and controls the new outgrowth a posterior end will arise, irrespective of whether the cut surface is anterior or posterior.

In the present series this 'inverse' or more properly differential effect of the KCN appears clearly only in the *c*-pieces, while in the *b*- and *a*-pieces a direct depressing effect appears. As a matter of fact, however, the two factors in the effect of the KCN, the direct and the differential, are always present and the actual result

in any given case depends on which of the two factors overbalances the other. In the *a*-pieces, for example, the differential effect does not play any important part in the total result; in the *b*-pieces the two factors are nearly balanced and in the *c*-pieces the differential effect overbalances the direct effect, except in the normal column and the result is an apparent morphogenetic stimulation by means of a depressing agent. It will be shown in later papers that the differences in the effect of the KCN at different levels of the body depend first upon the axial gradient and second upon the dynamic changes in the pieces following isolation.

Turning now to the *d*-pieces, we find in *IId* 10 per cent normal and 82 per cent teratophthalmic, a total of 92 per cent with eyes as against a total of 94 per cent with eyes in *Id*; but *IId* shows only 10 per cent normal as compared with 34 per cent normal in *Id*, and 82 per cent teratophthalmic, as compared with 58 per cent in *Id*. The KCN has changed 24 per cent of the pieces from normal to teratophthalmic. Here then the effect of the KCN is very similar to that in the *a*-pieces. The *d*-pieces form the anterior region of the second zoöid and it is clear that they show a marked difference in their reaction to the KCN from the *c*-pieces which form the posterior region of the first zoöid. In this series the remaining parts of the posterior zoöids were not included.

Series 419A 1 and 2.² November 2, 1911 to February 1, 1912. In this series the effects of different temperatures on regulation are compared. The worms were collected November 2, 1911, from spring water at 10° to 12°C. and after collection were kept in a refrigerator at 10° to 12°C. until December 23, when the pieces were cut. During this time the worms received maximal feeding, although in consequence of the low temperature their reaction to food was slight.

On December 23 lots of fifty pieces each of *a-d*, as indicated on the left side of figure 46, were cut in water at 10° to 12°C., but as

² This series as given here forms a part of a larger series on acclimatization to different temperatures and its physiological after-effects. The series as a whole shows very clearly that worms which are acclimated to different temperatures possess very different regulatory capacities at any given temperature. The results of this and other similar series give us some insight into the nature of the process of acclimatization.

soon as cut were placed in water at room temperature, 18° to 22°C. and allowed to regulate there. Similar lots of fifty pieces each were cut at 10° to 12°C. and then kept in water of the same temperature during regulation. The series is then a comparison of the character of regulation at two different temperatures of pieces of animals kept at the lower of the two temperatures before operation.

Table 6 gives the results in percentages: *II 1 a-d* are the pieces which regulate at the higher temperature, 18° to 22°C. and *II 2 a-e* those which regulate at 10° to 12°.

In *II 1* regulation was complete in less than two weeks and the pieces were examined on January 7, 1912: in *II 2*, on the other

TABLE 6

PIECES	NORMAL	TERATOPHTHALMIC	TERATOMORPHIC	ANOPHTHALMIC	HEADLESS	DEAD
<i>II 1 a</i>	86	14	0	0	0	0
<i>II 2 a</i>	24	76	0	0	0	0
<i>II 1 b</i>	56	42	2	0	0	0
<i>II 2 b</i>	0	40	6	52	2	0
<i>II 1 c</i>	10	62	14	8	4	2
<i>II 2 c</i>	0	14	2	30	50	4
<i>II 1 d</i>	78	22	0	0	0	0
<i>II 2 d</i>	8	64	14	12	2	0

hand, regulation was not completed before February 1. In this series, as in the KCN series, 432 above, the three pieces *a*, *b* and *c* are almost always within the first zooid while *d* almost always consists in large part of the anterior region of the second zooid.

At both temperatures the characteristic axial gradient appears, as table 6 shows. The regulatory capacity decreases from *a* to *c* and increases again in *d*. But comparison of the results at the two temperatures shows first that the pieces at the higher temperature possess a much greater regulatory capacity than those at low temperature, and, second, that the effect of the temperature differs in pieces from different levels. In the *a*-pieces 62 per cent are changed by the higher temperature from teratophthalmic to normal: in the *b*-pieces the difference is still greater, for at the higher temperature 56 per cent are normal and 100 per cent form

eyes, while at the lower temperature none are normal and only 46 per cent form eyes at all. In the *c*-pieces the difference is even greater than in the *b*-pieces: here $10 + 62 + 14$, 86 per cent in all form eyes at the higher temperature and only 12 per cent are anophthalmic or headless, 2 per cent having died: at the lower temperature $14 + 2$, 16 per cent form eyes, 80 per cent are anophthalmic or headless and 4 per cent have died. Moreover, at the lower temperature no normal and only 14 per cent teratophthalmic forms appear, as compared with 10 per cent normal and 62 per cent teratophthalmic at the higher temperature.

In the *d*-pieces, however, the temperature effect is again much less. At the lower temperature 8 per cent are normal, 64 per cent teratophthalmic and 14 per cent teratomorphic, a total of 86 per cent with eyes, while at the higher temperature 78 per cent are normal and 22 per cent teratophthalmic, a total of 100 per cent with eyes. This effect in the *d*-pieces is very similar to that in the *a*-pieces, although somewhat greater. In the *a*-pieces the changes all lie between teratophthalmic and normal eyes and in the *d*-pieces this is true for 72 per cent, only 28 per cent being shifted by the higher temperature from the teratomorphic, anophthalmic and headless columns to the teratophthalmic or normal. In the *b*-pieces, on the other hand, 54 per cent are shifted by the higher temperature from the anophthalmic and headless groups to the groups with eyes and in the *c*-pieces 68 per cent are shifted in the same way.

It is evident from this series, as from the KCN series above that a given external factor produces very different effects at different levels of the body and that a marked difference in the reaction of the posterior region of the first and the anterior region of the second zoöid to the factor concerned exists. In other words, the effect of a higher temperature in increasing the regulatory capacity of pieces of worms kept at low temperature before operation increases from the anterior to the posterior end of the first zoöid and is again much less in the anterior region of the second zoöid. The reasons for these differences will appear later: at present these data serve merely as evidence for the existence of a dynamic factor along the axis of the body, which shows not only a grada-

tion within the limits of a single zoöid, but also a marked change between the first and the second zoöids.

Many other series have been carried through with KCN, with alcohol and with low temperature and in all the region of the second zoöid differs in its reaction to the experimental factor from the posterior region of the first zoöid.

2. Some further cyanide experiments: the relation between method and results

It must not be supposed that a given experimental factor always produces the same results on animals or pieces which are in the same physiological condition. The character of the result depends to a large extent upon the method in which the experimental factor is used. Two series are presented to illustrate this point: in both the differences between first and second zoöids appear, but they appear in a different way because the KCN is used differently in the two series. These series and many others essentially similar to them constitute further evidence in support of the dynamic character of the axial factor. All that was said above (p. 136) concerning the size of the worms, their feeding, etc., applies to the two following series as well.

Series 512 I and III. March 5 to 18, 1912. In this series KCN $1:1000$ m. acting during the first twenty-four hours after the pieces were cut, is the experimental factor used. The whole length of the worms except the head was cut into six pieces as nearly as possible equal in length, as indicated on the *right* side of figure 46. Of these pieces *a* and *b* included most of the first zoöid, while *c* consisted of the posterior region of the first and the anterior region of the second zoöid and *d*, *e* and *f* were parts of the posterior zoöids, *f* corresponding more or less closely to the most posterior zoöid or the 'growing tip' (Child, '11 f): each piece is then somewhat longer than the pieces in the two series of the preceding section and the *c*-pieces include parts of both the first and second zoöids.

Lots of fifty each of these six pieces were placed in water at room temperature as a control. Similar lots, but of forty each instead of fifty—the stock from which these worms were taken

contained no more worms of the proper size—were prepared as follows: the worms were cut in water at room remperature and each piece as soon as cut was dropped into a liter Erlenmeyer flask full of KCN $\frac{1}{50000}$ m. After all pieces were cut the solution in the flasks was replaced by fresh of the same concentration and the flasks were corked. After twenty-four hours the pieces were rinsed and placed in water where they remained until regulation was complete. Table 7 gives the results in percentages, *Ia-Ij* being the controls in water and *IIIa-IIIj* the KCN lots.

In the control the axial factor appears as usual in *a* and *b*, but *c* shows only a slightly lower head and eye frequency than *b*.

TABLE 7

PIECES	NORMAL	TERATOPH- THALMIC	TERATO- MORPHIC	ANOPH- THALMIC	HEADLESS	DEAD
<i>Ia</i>	74	26	0	0	0	0
<i>IIIa</i>	52.5	47.5	0	0	0	0
<i>Ib</i>	2	78	2	14	4	0
<i>IIIb</i>	0	40	20	35	5	0
<i>Ic</i>	12	50	8	16	14	0
<i>IIIc</i>	7.5	45	5	20	17.5	5
<i>Id</i>	50	46	0	2	2	0
<i>IIId</i>	50	50	0	0	0	0
<i>Ie</i>	50	50	0	0	0	0
<i>IIIe</i>	47.5	47.5	2.5	2.5	0	0
<i>Ij</i>	100	0	0	0	0	0
<i>IIIj</i>	97.5	2.5	0	0	0	0

instead of much lower as in series 432 and 419 in the preceding section. This is because in the present series the *c*-pieces include a part of the second zooids, while in series 432 and 419 they were almost wholly within the first zooid.

The *d*- and *e*-pieces are almost exactly alike and show a much higher frequency of heads and eyes than the *c*-pieces. The similarity of the *d*- and *e*-pieces probably indicates that each of them belongs to a different zooid for if *d* were the anterior and *e* the posterior region of the same zooid the axial gradient would appear.

Probably then the region *c, d, e* as indicated on the right side of figure 46 consists of two zooids which have resulted from the phy-

siological division of one. And finally the *f*-pieces of the control are 100 per cent normal, a much higher percentage than in *d* or *e*. Figure 46 shows that these pieces correspond approximately to the most posterior zoöid or series of short zoöids and their peculiar regulatory capacity is due to this fact.

In the KCN pieces *IIIa* shows a slight decrease in normal forms as compared with *Ia*, and *IIIb* shows a much greater decrease in head frequency as compared with *Ib*. In *c*, *d*, *e* and *f*, on the other hand, the KCN has practically no effect on the character of the regulation, i.e., the posterior zoöids are clearly distinguishable from the posterior region of the anterior zoöid by the difference in their reaction to the KCN.

Series 494 I and II. February 15 to March 3, 1912. Here, as in the preceding series, the body is cut into six pieces of equal length and regulation in water is compared with regulation in KCN but the KCN is used in a different way. The worms for the KCN lots were placed in KCN $\frac{1}{20000000}$ for three to five minutes before being cut into pieces, the pieces were then cut in the same solution and were placed at once in flasks which also contained KCN of the same concentration: after forty-eight hours they were rinsed and returned to water where they remained until regulation was completed. Lots of fifty pieces each were used. The controls consisted of lots of fifty each in water at the same temperature.

TABLE 8

PIECES	NORMAL	TERATOPIH- THALMIC	TERATO- MORPHIC	ANOPH- THALMIC	HEADLESS	DEAD
<i>Ia</i>	78	22	0	0	0	0
<i>IIa</i>	62	38	0	0	0	0
<i>Ib</i>	0	90	2	4	2	2
<i>IIb</i>	8	92	0	0	0	0
<i>Ic</i>	10	76	0	6	8	0
<i>IIc</i>	60	34	0	4	2	0
<i>Id</i>	28	68	0	0	4	0
<i>IIId</i>	72	28	0	0	0	0
<i>Ie</i>	52	48	0	0	0	0
<i>IIe</i>	94	6	0	0	0	0
<i>If</i>	100	0	0	0	0	0
<i>IIIf</i>	98	2	0	0	0	0

Table 8 gives the percentages. *Ia-Ij* are the controls and *Ila-Ilf* the KCN-pieces.

A comparison of the controls in table 8 with those of table 7 (p. 144) gives some idea of the degree of uniformity of the results in parallel series. The worms for the two series were taken from the same stock about two weeks apart. A comparison of the two tables shows that the percentages are almost the same in both in the pieces *a*, *b*, *e* and *f*, while pieces *c* and *d* show differences of about 25 per cent in the two tables. These differences in *c* and *d* are correlated with the presence of the second zoöid. Slight differences in the levels of the cuts in relation to the boundaries of the zoöids account for all such differences. If my series consisted of much larger numbers of pieces these differences would be much less, but it is impossible to take two sets of fifty worms each, of approximately the same length and cut them into six pieces so that the levels of the cuts shall stand in the same mean relation to the boundaries of the zoöids in each set. The similarity of the other percentages in the controls shows, however, the value of the method in regions where an invisible and uncontrollable factor is not involved.

In the present series the effect of the KCN on the character of regulation is different in certain respects from that in series 512 above. In *Ila* a slight decrease in the frequency of normal forms appears, as compared with the control *Ia*. The *Ilb*-pieces show a slight increase in regulatory capacity over *Ib* and the pieces *Ilc*, *Ild*, *Ile* show a great increase over the controls. In the *f*-pieces the influence of KCN does not appear at all in the character of regulation, though of course the rate in *Ilf* is much less than that in *If*. No increase is possible here since the control shows 100 per cent of normal forms, so that all that we can say concerning the effect of the KCN on these pieces is that it does not decrease their regulatory capacity. In all probability its physiological effect is similar to that on the *d*- and *e*-pieces, but does not appear morphologically.

This series affords a good contrast with the preceding. Here the whole worms were placed in KCN before cutting, were cut in KCN and remained in KCN forty-eight hours: here the effect of

the KCN on regulation in the first zooid is very slight and in the posterior zooids the KCN increases the regulatory capacity except in the *IIf* pieces, where no morphological effect appears. In series 512 the pieces were placed in KCN of higher concentration as soon as cut and remained there for twenty-four hours. In this series the effect of the KCN is a decrease in the regulatory capacity in the first zooid and no change at all in the posterior zooids. In general the effect of the KCN is different in the two cases but the anterior and posterior zooids are clearly distinguishable in both by the difference in their dynamic condition. The question as to why the different use of KCN gives such different results will be considered elsewhere.

Such series as these suffice to distinguish the anterior zooid from the group of posterior zooids, but they do not give any exact information as to the number of posterior zooids or the position of their boundaries. To obtain more definite results along this line it is necessary to cut the region of the posterior zooids into much shorter pieces: when this is done differences similar to those between the first and second zooid in the above series though less marked, appear between the other zooids composing the posterior region: the anterior region of any zooid is in a different condition from its posterior region and from the posterior region of the zooid anterior to it. The only difficulty in the way of such experiments is the variation in level of the boundaries between zooids in different individuals. If for example, we cut the post-pharyngeal regions of fifty worms 18 mm. in length into ten pieces each and record the results for each lot of corresponding pieces, we obtain only vague indications of the levels of the different zooids because of the variations in level in different individuals. *If, on the other hand, we isolate each of the ten pieces from each worm and record results we shall find that they are much more definite for each individual and the variation in different individuals becomes apparent.

III. DISCUSSION

The experiments described above indicate that the processes concerned in morphogenesis, or at least some of them, differ in

certain respects at different levels of the body. The axial factor appears, not only in the regulatory development of the different organs of the single piece but in the regulation of pieces from different levels of the body.

In any single piece of *Planaria* which forms a new whole we can see that under natural conditions the regulatory development of the new posterior end—not merely the regeneration in the stricter sense but the whole development—is a slower process than the development of the head. Under conditions which decrease metabolism this difference becomes beyond a certain point not merely a difference in rate but a difference in capacity. Instead of forming a tail more slowly than a head the piece now forms no tail at all, but may still give rise to a head. A similar relation exists between head-formation and pharynx-formation and it has been shown above that the development of a posterior end may be largely or wholly inhibited without entirely preventing the formation of the pharynx. In every case the effect of the depressing factor on regulatory morphogenesis in a piece from a single zoöid increases posteriorly along the axis.

Moreover, the experimental data indicate as far as they go that this axial factor is essentially quantitative rather than qualitative. The rate of the dynamic processes or certain of them evidently decreases from the anterior end posteriorly along the axis and the apparent qualitative differences under experimental conditions are due merely to the fact that under these conditions the rate of reaction becomes so low that little or no morphological effect is produced. In other words, the evidence thus far points to the existence of an axial gradient in rate of reaction as the fundamental feature rather than a gradation of substances, such as Morgan and others have assumed to exist. It is probable that such a gradient can and does produce secondarily a localization of different substances or of different quantitative relations in a complex of substances at different levels along the axis.

The evidence for the existence of an axial gradient in rate of reaction presented in this paper is based on visible morphological features. In the following paper another line of evidence will

be presented which has to do with the physiological resistance of different levels of the body to certain agents and the relation between this resistance and the rate of reaction. We shall see that the axial gradient of resistance to anesthetics, KCN, etc., is similar to that of regulatory morphogenesis. In both cases the same processes are involved: in the one case we examine the morphological records of the processes, in the other we compare the processes and the results of the two methods confirm each other.

The experiments recorded in Section C show, however, that the axial gradient changes more or less abruptly between the posterior end of the first and the anterior end of the second zooid. As a matter of fact each zooid possesses a gradient of its own, though in the posterior zooid where the development is not advanced the individual gradients are much less sharply distinguishable from each other than in the case of the first and second zooids.

As regards the different effects of the KCN in the three series, nos. 432, 512 and 494 given in the tables in Section C, it is impossible to go into details until further data concerning the nature of the axial gradient and the dynamics of regulation are presented. It may be said, however, that all these differences are readily interpreted on the basis of the following facts which, as will be shown, are all well established: first, the axial gradient is a gradient in the rate of the dynamic processes and primarily of the oxidations, and the head region is dominant because it is the region of highest rate. Second, the physical isolation of a part increases temporarily its rate of reaction in direct proportion to the degree of its subordination to more anterior regions, in other words, the direct effect of isolation in accelerating the rate of reaction in a piece increases with increasing distance from the original head region. This holds only within the limits of a single zooid: the anterior region of each zooid is one in which physical isolation produces little or no acceleration of the rate of reaction and this is so because these regions are relatively independent of others, i.e., are dominant. Third, the formation of a new head in a headless piece is not in any sense a restitution of a missing part, but the formation of a new individual head first. The new head forms from the cells which are directly affected by the wound and the

absence of other cells anterior to them; whether a head shall form or not depends on whether the reaction in these cells proceeds far enough so that it becomes the controlling factor in the piece. If it becomes dominant to a sufficient degree a new head forms and the new head region determines the reorganization of other parts from the anterior end backward: if it does not become dominant a head does not arise. In the formation of a new head then the relation between two factors which are in a sense opposed to each other is involved. This relation differs in different regions of the body in consequence of the preëxisting axial gradient and the stimulating effect of isolation, moreover, it can be altered in various ways by the use of reagents and other external factors. Under certain conditions or in certain regions of the body depressing agents like KCN act as apparent morphogenetic stimuli simply because they alter the relations between the two opposed reaction-complexes of the piece in favor of the reaction-complex which leads to head-formation. In certain other regions or under other conditions they alter the relations in the opposite direction and so decrease the frequency of head-formation and consequently the capacity of the piece for regulation.

If these two opposed factors exist, as they undoubtedly do, then the regulatory capacity of a piece is not definitely and finally determined by its position in the original body, its relation to the axial gradient or its 'organization,' but by the relation of the two opposed factors to each other and it has been shown above that this relation can be altered experimentally.

It is true, however, that under constant conditions the relation of these two factors to each other is determined within certain limits of variation by the position of the piece within the body, but 'position' in this connection means essentially its relation to the dominant region, i.e., its position in the axial gradient, rather than a certain 'organization.' Since this is the case the regulatory capacity of pieces taken in sequence along the axis of the animal shows a gradient which is related to the axial gradient in the intact animal but is not necessarily a direct and simple expression of it. The fact that the regulatory capacity of pieces is not fixedly determined, but can be altered experimentally, in either

direction demonstrates clearly enough that the relation between this capacity and the axial gradient is not simple but complex.

The possibility of altering and controlling experimentally the regulatory capacity of pieces, not only as regards rate and size of parts but also as regards the presence or absence of their most important morphological characteristics, the head, the eyes, the auricles, the pharynx and the posterior end points to a promising field of investigation. Moreover, since the experimental factors which accomplish these results are such as influence the dynamic processes in the pieces and since in my own experiments it is known that their effect is primarily quantitative, it is not too much to say that these experiments throw some light on the problem of the dynamics of morphogenesis and inheritance. And finally it is evident that we must interpret regulatory phenomena in terms of dynamic processes rather than in terms of morphology.

IV. SUMMARY

1. When pieces of a single zoöid of *Planaria dorotocephala* undergo regulation in dilute anesthetics (alcohol, ether, chlorotone) the degree of retardation or inhibition of morphogenesis increases posteriorly along the axis of the piece. The formation of a head may occur under conditions which inhibit all other regulatory processes and the head and pharynx may form under conditions which inhibit the formation of the posterior end.

2. Very dilute solutions of KCN give results similar in general character to those obtained with alcohol, etc., but more striking in that the axial factor appears more clearly.

3. Experiments at different temperatures also show the existence of the axial factor, though less clearly than the anesthetics and KCN. Starvation and the presence of metabolic products of *Planaria* in the water likewise give essentially similar results.

4. The axial gradient also appears in the different effects of KCN and other depressing agents upon the process of head-formation at different levels of the body. The effect of the depressing agent is not only different in degree at different levels but under certain conditions may be different in direction. Cyanide,

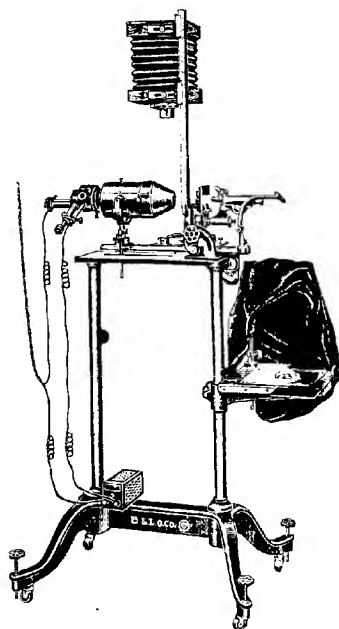
for example, may either decrease or increase the regulatory capacity of pieces according to the region of the body concerned and the method in which it is used. These opposite effects of the same reagent used in the same concentration are due to the fact that the process of head-formation in any given piece is the resultant of two opposed factors and the cyanide or other depressing agent may alter the relation between these two factors in either direction.

5. The axial gradient is not continuous and uniform from one zoöid to another, but each zoöid possesses an axial gradient of its own. The regulation of pieces in dilute KCN or other depressing agents shows that the anterior region of the second zoöid is in a different dynamic condition from the posterior end of the first. In general the same is true for any two zoöids, but in the posterior zoöids which are only slightly developed, the differences between the posterior end of one and the anterior end of another are often slight.

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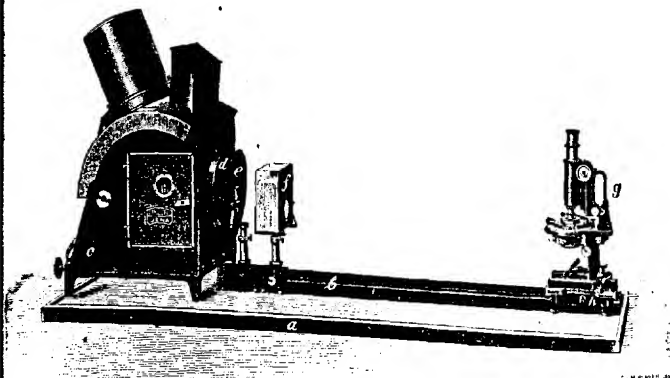
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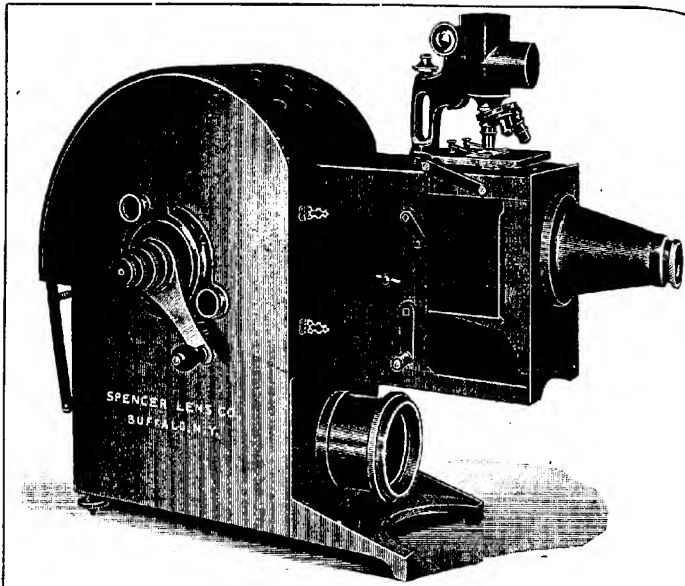
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THE MODE OF INHERITANCE OF FECUNDITY IN THE DOMESTIC FOWL¹

RAYMOND PEARL

THREE FIGURES

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¹ Papers from the Biological Laboratory of the Maine Experiment Station No. 37. An abstract of this paper was presented at the meeting of the American Society of Naturalists in Princeton, N. J., December, 1911.

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INTRODUCTION

During the course of an investigation into the inheritance of fecundity in the domestic fowl, which has now involved thirteen generations and several thousand individuals, and has occupied the major portion of the writer's time during the past five years, two definite and clear-cut results have come to light.² These are:

First: that the record of egg production or fecundity of a hen is not of itself a criterion of any value whatsoever from which to predict the probable egg production of her female progeny. An analysis of the records of production of large numbers of birds shows beyond any possibility of doubt that, in general, there is no correlation between the egg production of individuals and either their ancestors or their progeny.

Second: that, notwithstanding the fact just mentioned, fecundity is, in some manner or other, inherited in the domestic fowl. This must clearly be so, to mention but a single reason, because it has been possible to isolate and propagate from a mixed flock

² For a complete list to date of the publications, in which the results of the investigation referred to have appeared, see the bibliography at the end of this paper. Throughout this paper numbers in parentheses refer to titles in the bibliography.

'pedigree lines' or strains of birds which breed true, generation after generation, to definite degrees of fecundity. Some of these lines breed true to a high condition or degree of the character fecundity; others to a low state or degree of this character.

Definite as these results are they give no clue as to how fecundity is inherited; what the mechanism is. Plate (43) has recently said: "Das Ziel der Erbliehkeitsforschung muss die Aufstellung von 'Erbformeln' für alle untersuchten Merkmale sein." This expresses the case precisely. To determine the 'Erbformeln' of fowls with respect to fecundity has been the goal towards which every part of the present investigation has been directed and urged. It is believed that a first approximation to the solution of the problem has now been reached. While there remain obscure points still to be cleared up, yet the results now in hand appear to indicate pretty clearly the general character of the mechanism of the inheritance of fecundity, and to show what lines further investigation of the problem may most profitably take. It is the purpose of this paper to present an account of the results mentioned. In doing this it will be necessary to bring forward evidence of several distinct sorts, anatomical and physiological as well as genetic. Only by approaching this problem of the inheritance of fecundity from all angles has it been possible to gain that understanding of the character itself which, in this instance certainly, is absolutely essential to a correct interpretation of any results respecting its inheritance.

BIOLOGICAL ANALYSIS OF THE CHARACTER FECUNDITY

At the outstart it will be well to understand clearly what is meant by the term fecundity as here used. In a former paper (34) the terms 'fecundity' and 'fertility' were defined as follows, and have been used as there defined throughout the course of the investigation.

We would suggest that the term 'fecundity' be used only to designate the innate potential reproductive capacity of the individual organism, as denoted by its ability to form and separate from the body mature germ cells. Fecundity in the female will depend upon the production of ova and in the male upon the production of spermatozoa. In mam-

mals it will obviously be very difficult, if not impossible, to get reliable quantitative data regarding pure fecundity. On the other hand we would suggest that the term 'fertility' be used to designate the total actual reproductive capacity of pairs of organisms, male and female, as expressed by their ability when mated together to produce (i.e., bring to birth) individual offspring. Fertility, according to this view, depends upon and includes fecundity, but also a great number of other factors in addition. Clearly it is fertility rather than fecundity which is measured in statistics of birth of mammals.

Taking fecundity as above defined it is obviously a character depending upon the interaction of several factors. In the first place the number of ova separated from the body by a hen must depend, in part at least, upon an anatomical basis, namely, the number of ova present in the ovary and available for discharge. Further there must be involved a series of physiological factors. The mere presence of an anatomically normal reproductive system, including a normal ovary with a full complement of ova, and a normal oviduct, is not enough to insure that a hen shall lay eggs, that is, exhibit actual as well as potential fecundity. While comparatively very rare, cases do occur in which a bird possesses a perfect ovary and perfect oviduct and is in all other respects entirely normal and healthy, yet never lays even a single egg in her life time. Such cases as these prove (a) that what we may call the anatomical factor is not alone sufficient to insure that potential fecundity shall become actual, and (b) that the anatomical and physiological factors are distinct, in the sense that the normal existence of one in an individual does not necessarily imply the co-existence of the other in the same individual.

A case of this kind is found in hen no. 8051 hatched March 29, 1909, and killed for autopsy record August 24, 1911. This bird had the secondary sexual characters of the female perfectly developed, and was entirely normal in other respects (body weight, 2366 grams). This bird never laid an egg during its life. The ovary was normal (fig. 1) and was of about the size proper to a fully developed pullet just reaching the point of beginning to deposit yolk rapidly in certain oöcytes in preparation for laying. While counts were not made this ovary appeared to carry a normal number of oöcytes. In general it was anatomically normal,

but physiologically in the state of development appropriate to a five or six months old pullet just about to lay. The same was true of the oviduct.. In this case the physiological factor or factors necessary to the bringing about of ovulation were simply totally lacking, in an otherwise perfectly normal bird.

Some other cases demonstrating the same thing might be cited from our records, but this will suffice for present purposes.

Turning now to the physiological factors involved in fecundity it would appear that there are at least two such factors or groups

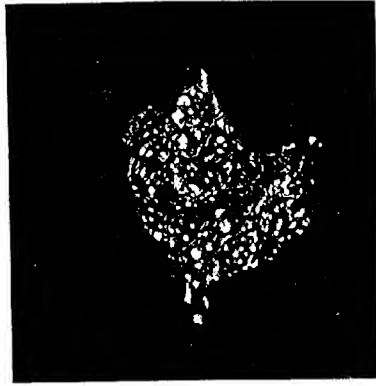


Fig. 1 Photograph (about twice natural size) of ovary of hen no. 8051. Note the presence of a large number of oocytes; none of which is enlarging in preparation for laying. See text for further explanation.

of factors. The first of these may be designated as the 'normal ovulation' factor. By this is meant the complex of physiological conditions which taken together determine the laying of about such a number of eggs as represents the normal reproductive activity of the wild *Gallus bankiva*. Under conditions of domestication the activity of this normal ovulation factor will mean the production of more eggs than under wild conditions. Continued egg production involves certain definite and rather severe metabolic demands, which under wild conditions will not always, or

even often be met. Further, as has been especially emphasized by Herrick (18, 19, and other papers), egg laying in wild birds is simply one phase of a cyclical process. If the cycle is not disturbed in any way the egg production is simply the minimum required for the perpetuation of the race. If, however, the cycle is disturbed, as for example, by the eggs being removed from the nest as fast as they are laid, a very considerable increase in the total number of eggs produced will result. This, of course, is what happens under domestication. What an effect in increasing the actual expressed fecundity of a wild bird the simple removal of eggs as fast as they are laid may have, may be illustrated by three cases from the literature. Austin (1) shows that whereas the wild Mallard duck in a state of nature lays only 12 to 18 eggs in the year, it will lay from 80 to 100 if they are removed as fast as laid and the bird is kept confined in a pen at night. Hanke (16) by regularly removing the eggs got 48 in succession from a common wryneck (*Inyx torquilla*³). Wenzel (53) in the same way brought a house sparrow's productivity up to 51 eggs.

With the domesticated *Gallus* the 'normal ovulation' factor may be taken as inducing a production of anything up to from forty to eighty eggs in a year, this production being spread over the period of from sometime in February to September or October. In this physiological complex are involved the elaboration and deposition of yolks, the rapid growth of a few oöcytes just preceding ovulation, ovulation itself, the activation of the oviduct, etc. The details of some of the processes involved have been described elsewhere (cf. Rubaschkin (44), Sonnenbrodt (48), Pearl and Curtis (33) and Pearl and Surface (37)) and do not concern us here. The essential point to be noted is that in this normal ovulation factor we are dealing with the basic physiological processes of normal 'unimproved' laying. To make a normal laying hen it is necessary to have present both the anatomical basis

³ I give this scientific name with much hesitation, not knowing what pranks the rule of priority or other nomenclatorial disturbers of the peace may have played with it in recent years. In any event the common name will quite sufficiently indicate what bird it is that is here under discussion.

discussed above and the physiological basis, which has been designated the normal ovulation factor.

It is a fact well known to poultrymen, and one capable of easy observation and confirmation, that different breeds and strains of poultry differ widely in their laying capacity. In saying this the writer would not be understood to affirm that a definite degree of fecundity is a fixed and unalterable characteristic of any particular breed. The history of breeds shows very clearly that certain breeds now notably poor in laying qualities were once particularly good. One of the best examples of this is the Polish fowl. But, in spite of this, inheritable breed and strain differences in fecundity exist, and probably always have existed. Such inheritable differences are independent of feeding or any other environmental factors. Thus the strain of Cornish Indian Games with which I have worked are poor layers, regardless of how they are fed or handled. This is merely a statement of particular fact; it does not imply that there may not exist other strains of Cornish Indian Games that are good layers.

The difference between this strain of Cornish Indian Games and Barred Plymouth Rocks, when kept under the same conditions and managed in the same way, is shown in tables 1 and 2, which give the frequency distributions and constants respectively, for flocks of these breeds kept at the Maine Station. The birds included in table 1 were all pullets, hatched at approximately the same time, and reared, housed, fed and cared for in all respects similarly. The Plymouth Rock distribution includes birds of both high and low fecundity strains. The low producing birds lower the mean in what is really an unfair manner, so far as concerns breed comparisons. The point is that, in the work of the Station, low-producing lines have been propagated for experimental purposes to a much greater extent than would be the case in purely random breeding of the Maine Station's stock, the Barred Plymouth Rock breed. To make a perfectly just comparison between Cornish Indian Games and Barred Rocks, the strains of the latter deliberately bred for low egg production should be excluded. It has, however, in the present case been

TABLE 1

Frequency distribution of winter egg production of the Barred Plymouth Rock and Cornish Indian Game breeds

EGGS LAID IN THE WINTER PERIOD	BARRED PLYMOUTH ROCKS LAYING THE SPECIFIED NUMBER OF EGGS		CORNISH INDIAN GAMES LAYING THE SPECIFIED NUMBER OF EGGS	
	Absolute number	Per cent of flock	Absolute number	Per cent of flock
0-5	43	14.4	32	48.5
6-11	22	7.4	8	12.1
12-17	28	9.4	9	13.6
18-23	19	6.3	6	9.1
24-29	25	8.4	7	10.6
30-35	26	8.7	1	1.5
36-41	19	6.4	3	4.5
42-47	27	9.0		
48-53	16	5.4		
54-59	21	7.0		
60-65	14	4.7		
66-71	10	3.3		
72-77	9	3.0		
78-83	3	1.0		
84-89	3	1.0		
90-95	0			
96-101	8	2.6		
102-107	0			
108-113	4	1.3		
114-119	2	0.7		
Total.....	299	100.0	66	99.9

TABLE 2

Constants for variation in winter egg production of the Barred Plymouth Rock and Cornish Indian Game breeds

BREED	MEAN	STANDARD • DEVIATION	COEFFICIENT OF VARIATION
	eggs	eggs	per cent
Barred Plymouth Rock.....	36.35 \pm 1.04	26.69 \pm 0.74	73.42 \pm 2.92
Cornish Indian Game.....	11.64 \pm 0.88	10.61 \pm 0.62	91.15 \pm 8.73
Differences.....	+24.71 \pm 1.36	+16.08 \pm 0.97	-17.73 \pm 9.21
Barred Plymouth Rock:			
All High Lines in 1908-09 ¹	54.16		
All High Lines in 1909-10 ¹	47.57		
All High Lines in 1910-11 ¹	50.58		

¹ Figures taken from Pearl (28).

deemed best to take the whole flock of Barred Rock pullets for the laying year 1910-11, without any selection. The comparison is sufficiently striking even on this basis.

From tables 1 and 2 it will be noted that:

1. The mean winter production of the Cornish Indian Games is less than one-third that of the general flock of Barred Plymouth Rocks, under uniform environmental conditions.

2. The winter production of the Games is considerably less than a fourth of that of the high producing lines of the Barred Rocks.

3. The variabilities in both cases are high, but relatively not significantly different. It is of interest to note that the observed coefficients of variation for winter production here given are of the same order of magnitude as the mean coefficients for the laying of the four winter months, November, December, January and February. Taking the mean of the coefficients of variation for these four months as given by Pearl and Surface (37, table 5, p. 96) we get 95.15.

The inferiority in egg production of the Cornish Indian Games is most strikingly shown by the integral curves from table 1. In table 3 the integral curves are given (in inversed form) for the winter production of Barred Rock and Cornish fowls.

The data of table 3 are shown graphically in figure 2.

This diagram is to be read in the following manner. The percentages of the flock laying a specified number of eggs are plotted on the abscissal axis. The different egg productions are plotted as ordinates. From the diagram it appears (for example) that whereas 47 out of every 100 birds in the Barred Rock flock each produced 35 or more eggs in the winter period, only 4 and a fraction birds out of every 100 in the Cornish Indian Game flock were able to produce as many eggs as this—35—in the same period.

Now in individuals which are high layers, and have this characteristic in hereditary form, there must be involved some further physiological factor in addition to the normal ovulation factor already discussed. An analysis of extensive statistics has shown (36, 37) that high fecundity represents essentially an addition of two definite seasonal, laying cycles to the basic, normal reproduc-

TABLE 3

Showing the percentage of the whole flock producing in the winter period more than certain specified numbers of eggs, in the case (a) of Barred Plymouth Rocks and (b) of Cornish Indian Games

THE INDICATED PERCENTAGE OF THE FLOCK PRODUCES IN THE WINTER PERIOD	BARRED PLYMOUTH ROCK	CORNISH INDIAN GAME
6 or more eggs.....	85.6	51.5
12 or more eggs.....	78.2	39.4
18 or more eggs.....	68.8	25.8
24 or more eggs.....	62.5	16.7
30 or more eggs.....	54.1	6.1
36 or more eggs.....	45.4	4.6
42 or more eggs.....	39.0	0
48 or more eggs.....	30.0	0
54 or more eggs.....	24.6	0
60 or more eggs.....	17.6	0
66 or more eggs.....	12.9	0
72 or more eggs.....	9.6	0
78 or more eggs.....	6.6	0
84 or more eggs.....	5.6	0
90 or more eggs.....	4.6	0
96 or more eggs.....	2.0	0
102 or more eggs.....	2.0	0
108 or more eggs.....	0.7	0
114 or more eggs.....	0.7	0
120 or more eggs.....	0	0

tion cycle. These added periods of productivity are what may be called (cf. 37, 28, 30) the winter cycle and the summer cycle. The winter cycle is the more important of these. It is the best practical measure of relative fecundity which we have and has been used as the chief unit of fecundity in these studies. It constitutes a distinct and definite entity in fecundity curves. The existence of this added fecundity, in high laying birds must depend upon some additional physiological factor or mechanism besides that which suffices for the normal reproductive egg production. Given the basic anatomical and physiological factors the bird only lays a large number of eggs if an additional factor is present.

As to the nature of this physiological mechanism we can only speculate. It probably involves fundamentally such matters as

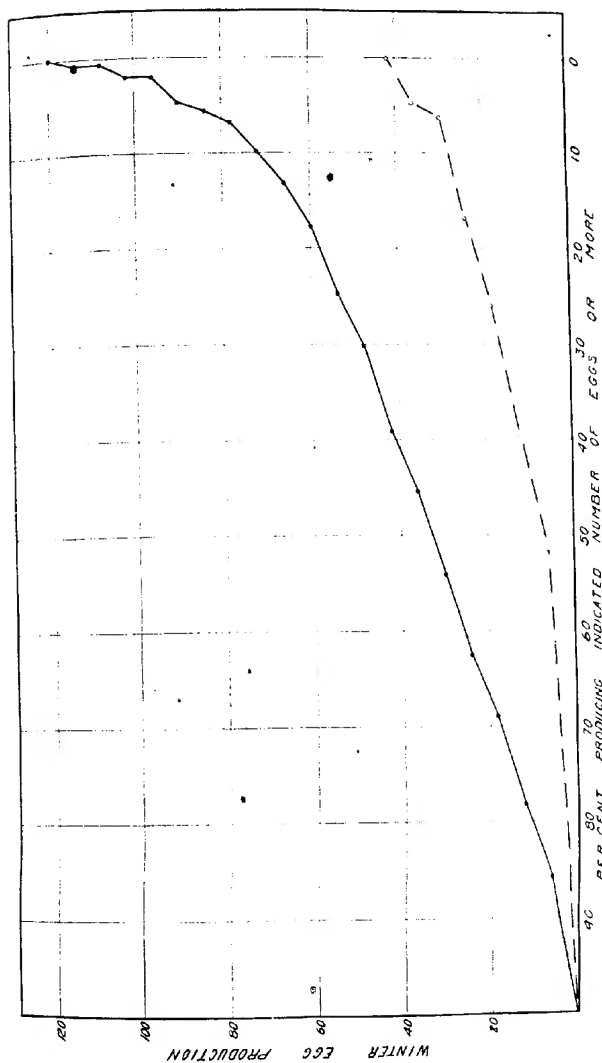


Fig. 2 Integral curves of winter egg production of Barred Plymouth Rock and Cornish Indian Game pullets. Solid line—Barred Plymouth Rocks. Broken line—Cornish Indian Games.

more perfect metabolism, including the distribution of substance and energy to the ovary, on which very heavy demands are laid in a high fecundity record. Immediately it involves a control of the process by which the supply of oöcytes on the ovary in the final stages of rapid growth by yolk deposition is kept at a relatively high level for long periods of time. Sonnenbrodt's (48) work suggests that the interstitial cells of the ovary may be connected with the process. Thus he says (*loc. cit.*, p. 421): "Bei älteren Hühnern findet man die Zwischenzellen immer noch, und besonders in der Nähe der Gefässe. Sie liegen heur gruppen-und nesterweise zwischen den Follikeln und *vor allem auch in den Stielen der grösseren Follikel*,⁴ immer dort, wo besonders starke Blutzufuhr günstige Ernährungsbedingungen biëtet."

It is quite conceivable that the presence of numerous interstitial cells on the stalks of the follicles of rapidly growing oöcytes is a cause of the rapid growth rather than an effect, as Sonnenbrodt suggests. The whole subject of the intimate physiology of the ovary needs more study.

Whatever the precise nature of the factor under discussion, which is a matter for future investigation, the main points which appear clear at present are that: (a) high fecundity represents a definite addition to the normal egg production sufficient in amount for purposes of reproduction. This added fecundity has been shown (*cf.* 23, 30) to be definitely inherited in certain cases at least and may be regarded as dependent on or determined by some physiological factor or complex of factors not present in birds which exhibit a low degree of fecundity.⁵ This physiological complex may be designated as the 'excess production' factor in fecundity.

We may next consider in greater detail these factors influencing fecundity, taking first

⁴My italics.—R. P.

⁵Throughout this discussion it is presumed that the reader will understand without repeated specific statements that attention was paid to environmental factors in the experimental work. That is, when the statement is made that one bird or set of birds exhibits high fecundity and another low fecundity it is to be understood that both sets were hatched, reared, fed and cared for in all respects in as nearly precisely the same way as is possible, considering that fowls are, in some degree, free agents and cannot be absolutely controlled. The extent both in time and

The anatomical basis of fecundity

*Since, as already pointed out, egg production obviously depends in part upon the presence of ova in a normal ovary, a question which demands consideration is the following:

To what extent are observed variations in fecundity (i.e., in the number of eggs laid) to be referred to anatomical differences? In other words, does the ovary of a high producing hen, with for example, a winter record of from 75 to 115 eggs, contain a larger number of oöcytes than does the ovary of a hen which is a poor producer, laying no eggs in the winter period and perhaps but 10 or 15 eggs in the year?

To get light upon this question the observations to be described have been made. The object was to arrive at as accurate a relative judgment as possible regarding the number of oöcytes in the ovaries of different individual birds. It is, of course, impossible practically to determine accurately the total absolute number of oöcytes in the ovary. What can be done, is to count the number of oöcytes which are visible to the unaided eye. While such results do not tell us, nor enable us to estimate with great accuracy, the total number of oöcytes in the ovary, they do nevertheless throw interesting and useful light on the question raised above.

The counts of the visible oöcytes for a number of birds are given in table 4. These counts were made at my suggestion by my assistant, Miss Maynie R. Curtis, to whose painstaking care and skill in carrying through the tedious business of counting it is a pleasure to acknowledge gratefully my indebtedness. Prof. W. F. Schoppe of the University of Maine is carrying this work forward and later we hope to be able to publish more extensive data.

space, and the manifoldness in respect to method, of the experiments upon which this discussion is based are so great and the checks on this point have been so numerous as to make it quite certain that the results are not influenced by a differential effect of the environment, arising from individual preferences of birds for particular sorts of food, or other similar peculiarities of behavior. When a result is stated to be due to inheritance the reader may assume, even though a specific statement is not made to that effect, that careful, critical consideration has been given to possible environmental influences.

So far as I am aware the counts here given are the first attempt yet made at anything more than the roughest sort of a guess at the number of eggs in a bird's ovary. While these counts do not give the total numbers they do establish minimum values. A given ovary certainly does not carry any less than the number of visible ova.

A word should be said as to the method of making the counts, and the meaning of the subdivisions of the table. The counts were made in some cases on fresh, and in other cases on preserved ovaries. There was found to be little difference in the two methods, as regards the ease and accuracy of counting. In making the counts small pieces of ovary were cut off, and teased apart with needles under water and the visible oöcytes on the small fragments counted. In delimiting boundaries where a number of small oöcytes were closely packed together, a hand lens was used. No oöcyte was counted, however, which could not be seen with the unaided eye. In other words the lens was not used to find oöcytes which might otherwise be missed, but merely to aid in the dissecting of the material.

In the oöcyte counts given in the table it will be noted that these are grouped into four categories. The first class includes ruptured follicles from which the ova have been discharged. A ruptured follicle which is large at the moment the ovum leaves it gradually shrinks in size and is more or less completely absorbed. On the ovary of a hen which has laid, however, there will always be found a certain number of these discharged follicles not yet absorbed. When such follicles get very small it is exceedingly difficult to distinguish them from small oöcytes (i.e., undischarged follicles). Undoubtedly there are errors in classification in this respect in the counts, but for present purposes this is not a matter of great importance. If the eye were sharp enough it might perhaps be possible to distinguish a ruptured follicle for every egg which has ever been laid, since it is doubtful if the absorption is ever so complete as to leave absolutely no scar. It is of interest to note that in the counts there is a reasonably close relation between the follicle count and the record of eggs laid.

The oöcytes proper are divided in the counting into three classes: those 1 cm. or over in diameter, those between 1 mm. and 1 cm. in diameter, and those less than 1 mm. in diameter. The first of these classes includes the large yolks nearly ready to leave the ovary and pass into the oviduct. They are in process of rapid enlargement by the deposition of yolk. The next class includes those oöcytes in which yolk deposition is started but is proceeding at a slow rate. It is from this class that the first class of rapidly growing yolks is constantly being recruited. Finally the "under 1 mm." class represents the make-up of the bulk of the ovary. It will be understood that these size classes are only roughly delimited, the diameter of each oöcyte having been estimated but not carefully measured.

Columns in the table are devoted to "Total number of eggs laid in life" and "Winter production." The first of these has no particular significance since obviously it depends on when the bird was killed in order to make the oöcyte count. Winter production, however, represents a definite entity in fecundity as already pointed out above (p. 162).⁶ Winter production records are directly comparable with one another. It is the inheritance of this fecundity unit that is primarily being studied in these investigations.

From this table a number of points are to be noted. In the first place it is clear that the number of visible oöcytes in the ovary of a hen is very large; much larger, I think, than has generally been supposed. While to be sure there are for the most part only vague statements respecting this point in the literature, usually these statements are to the effect that the bird's ovary contains 'several hundred' ova. The only direct statement as to the actual number of oöcytes in a hen's ovary which I have been able to find is given by Matthews Duncan (8) on the very dubious authority of Geyelin (11) to the following effect (*loc. cit.*, p. 36): "It has been ascertained that the ovarium of a fowl is composed of 600 ovula or eggs; therefore, a hen during the whole of her life

⁶ For general discussion of "winter production" as a unit of fecundity, see (28), (30), (31), (37), (38). It comprises the egg production up to March 1 of the laying year.

TABLE 4
Showing the number of visible oöcytes in the ovary of certain birds

CAGE NO.	BIRD NO.	BREED	DATE OF HATCHING	DATE KILLED	TOTAL NUMBER OF EGGS LAID IN LIFE	WINTER PRODUCTION	DISCHARGED FOLLICLES	OÖCYTES 1 CM. OR OVER IN DIAMETER	OÖCYTES 1 MM. TO 1 CM. IN DIAMETER	OÖCYTES UNDER 1 MM. IN DIAMETER	TOTAL VISIBLE OÖCYTES
1	8021	Barred Ply. Rock	June 1, '10	March 28, '11	10	3	17	9	53	1149	1228
2	8017	Barred Ply. Rock	June 2, '10	March 30, '11	10	0	12	7	51	1506	1606
3	8030	Barred Ply. Rock	June 1, '10	March 10, '11	7	0	8	5	62	830	914
4	8005	Barred Ply. Rock	June 2, '10	March 14, '11	17	5	12	8	68	1006	1174
5	1367	Barred Ply. Rock	April 28, '10	April 4, '11	34	3	49	7	29	2121	2305
6	8018	Barred Ply. Rock	June 2, '10	March 24, '11	16	0	23	6	42	1123	1194
7	8009	Barred Ply. Rock	June 2, '10	March 24, '11	15	0	17	6	49	2029	2101
8	8010	Barred Ply. Rock	May 19, '10	March 17, '11	19	5	24	5	92	1455	1576
9	425	Barred Ply. Rock	March 30, '09	July 7, '10	23	0	21	12 ¹	142	1346	1521
10	3546	White Leghorn ²	May 18, '09	December 20, '10	198	51	75	3	231	2146	2452
11	2067	White Leghorn	May 28, '09	December 15, '10	197	32	217	1	108	3279	3605
12	3453	White Leghorn	May 21, '09	December 13, '10	10	0	11	3	75	1026	1701
13	3833	White Leghorn	June 14, '09	December 22, '10	2	0	43	3	80	2022	2145
14	52	Cornish Ind. Game	April 21, '09	July 12, '10	52	13	54	6	167	1823	1950
15	71	F ₁ Cross	March 31, '10	March 20, '11	124	103	50	5	70	1875	2000
16		Guinea hen	?	January	?	?	9	3	36	717	765
17		Guinea hen	?	January	?	?	3	3	38	545	586

¹ This includes 8 yolks in process of absorption.

² For this and the three following birds I am indebted to Prof. James E. Rice of Cornell University, who very kindly gave me these trap-nested individuals for use in the present study. The egg records in these cases are not the records for life, but the records up to November 1, 1910. The figures represent practically the total production.

³ Birds not in laying condition when killed.

cannot possibly lay more eggs than 600, which in a natural course are distributed over nine years in the following proportion." This statement is followed by an utterly preposterous and presumably entirely imaginary table from Geyelin, supposed to show the laying of hens at different ages. How far from the truth the table is is indicated by the fact that according to it the pullet year is the least productive of any of a hen's life, save only for the ninth year when the last remnants of the original 600 eggs are being tardily and, one must suppose, sorrowfully ejaculated!⁷ As a matter of fact repeated trap-nest and other tests in all parts of the world have shown again and again that, on the average, the pullet year is the most productive of a hen's life.

From the figures given in table 4 it is furthermore apparent that the absolute number of oöcytes in the hen's ovary is very much larger than the number of eggs which any hen ever lays. A record of 200 eggs in the year is a high record of fecundity for the domestic fowl, though in exceptional cases it may go even a hundred eggs higher than this (cf. 29). But even a 200-egg record is only a little more than a tenth of the average total number of visible oöcytes in a bird's ovary, to say nothing of the probably much larger number of oöcytes invisible to the unaided eye, but capable of growth and development. In other words it is quite evident from these figures that the potential 'anatomical' fecundity is very much higher than the actually realized fecundity. This is true even if we suppose the bird to be allowed to live until it dies a natural death. Experience shows that birds which make a high fecundity record in the first year of their life, generally speaking, never do so thereafter. In general an examination of what long period records are available in the statistics of this Station, and also in the literature, indicates that probably only relatively few birds of the American or Asiatic breeds at least, would lay many more than 400 to 500 eggs in their natural life time, if they were allowed to live it out. Records of '1000-egg' birds are in existence, but such birds are rare.

⁷ It is difficult to understand how so acute an investigator as F. H. A. Marshall could have been so imposed upon by this wonderful table of Geyelin's as to republish it in his valuable and interesting book on the "Physiology of Reproduction."

One of the longest continuous egg records of an individual bird, which may be considered accurate, with which I am acquainted is that given by Handrik (15) (for a Leghorn). This bird was hatched in 1901. Its egg record was as follows:

<i>Calendar year</i>	<i>Eggs laid</i>
1902	105
1903	163
1904	138
1905	159
1906	160
1907	133
1908	111
Total.....	969
Average per year.....	138 $\frac{3}{4}$

Heier (17) gives a four-year record for a Braekel hen, which is distinctly higher than would usually be obtained over so long a period. The figures are as follows:

<i>Laying Year</i>	<i>Eggs laid</i>
First.....	153
Second.....	139
Third.....	152
Fourth.....	162
Total.....	606
Average per year.....	151 $\frac{1}{2}$

In this connection the paper of Dackweiler (5) is of interest. Both of the cases here cited are of fowls of the Mediterranean type, in which the tendency to accumulate body fat with advancing age is not marked. I know of no records comparing with these in extent for Plymouth Rocks or other American or Asiatic breed. After two years the fecundity of Plymouth Rocks, in all cases which have been observed at the Maine Experiment Station, becomes greatly reduced.

An examination of table 4 in detail indicates that there is no very close or definite relationship between the number of visible number of oöcytes on the ovary and the winter production of a bird. Thus no. 1367 and no. 8546 each have about the same

number of visible oöcytes, yet one has a winter production record 18 times as great as the other. Again no. 71 with the extraordinarily high winter record of 106 eggs has only a little more than one-half as many visible oöcytes as has no. 2067, whose winter production record is only 32 eggs. Again no. 71 with its 106 record has very nearly the same oöcyte count as no. 8010 with a winter record of zero. In general it may be said that the present figures give no indication that there is any correlation between fecundity as measured by winter production, and the number of oöcytes in the ovary. Of course, the present statistics are meager. More ample figures are needed (and are being collected) from which to measure the correlation between actual and 'anatomical' fecundity.

But the data now in hand, even at the very lowest valuation which may be placed upon them, indicate clearly, it seems to me, that there must be some other factor than the anatomical one involved in the existence of different degrees of actual fecundity in the domestic fowl. It clearly is the case from table 4 that when one bird has a winter record of twice what another bird has it is *not* because the first has twice as many oöcytes in the ovary. On the contrary it appears that all birds have an anatomical endowment entirely sufficient for a very high degree of fecundity, and in point of fact quite equal to that possessed by birds which actually accomplish a high record of fecundity. Whether or not such high fecundity is actually realized evidently depends then upon the influence of additional factors beyond the anatomical basis. As has already been indicated in the preceding section it is reasonable to suppose that these factors are physiological in nature. The record of hen no. 71 shows most clearly and distinctly the reason why we must assume that there are definite physiological factors at work in determining relative degrees of fecundity, as measured by winter production.

While there are no oöcyte counts yet available for wild birds it is possible that when made they will show the same point as is here brought out, namely that there is no close or definite relation between the anatomical endowment and actually realized fecundity. In this connection a statement made by Jenner (20)

a century and a quarter ago regarding the cuckoo is of interest. He says:

That the cuckow actually lays a great number of eggs, dissection seems to prove very decisively. Upon a comparison I had an opportunity of making between the ovarium, or racemus vitellorum, of a female cuckow, killed just as she had begun to lay, and of a pullet killed in the same state, *no essential difference appeared*.⁸ The uterus of each contained an egg perfectly formed and ready for exclusion; and the ovarium exhibited a large cluster of eggs, gradually advanced from a very diminutive size to the greatest the yolk acquires before it is received into the oviduct.

The mechanism of the inheritance of fecundity

With so much by way of introduction we may proceed to the subject in hand, namely a detailed account of the manner in which fecundity is inherited. In this account for reasons which have been stated above, and in earlier papers on this subject, attention will be confined to winter egg production.

A. *Observed types of winter egg production.* A study of numerous statistics shows that hens fall into three well defined classes in respect to winter production. These classes include (a) those birds which lay no eggs whatever in the winter period (up to March 1 of the laying year); (b) those that lay but have a production during the period of something under about 30 eggs; and finally (c) those whose production exceeds 30 eggs in the winter period. The division point between classes (b) and (c) is not sharply defined in every case, but it is plainly (as will appear later) at about 30 eggs. Since in the analysis some fixed point must be taken for this boundary a production of 30 has been chosen for this purpose and will be used throughout. This is an arbitrary choice only in the sense that it is a convenient round number lying near where the biological division point falls, at least in the strains of domestic fowls used in these experiments. The analysis could doubtless be carried through nearly as well by taking the division point at a production of 29 or 31, but 30 is a more convenient figure.

⁸ Italics not in original.

In making the division of winter egg production into three groups it must be remembered that this is a character subject to purely somatic fluctuations and environmental influence. Allowance for these factors must be made in interpreting and classifying results. In particular the following points must be kept in mind throughout.

(1) A zero winter production may be due to genetic causes or to purely somatic (physiological) ones, and there is nothing in a single record of this sort, taken by itself, to indicate to which category it belongs. A bird may carry the factor or factors for winter production, yet owing to purely physiological causes, such as a disturbance of metabolism, or of the ovary in respect to its physiology, or to disease, patent or obscure, it may never actually lay during the winter period. Usually it will be possible to tell from other considerations than the record itself, whether a given zero record is 'somatic' or 'genetic.'

(2) The upper limit of the winter period at March 1 is arbitrary, and only approximately coincides with the biological reality. Actually with most birds the spring or reproductive cycle of production (cf. 37) begins in the latter part of February. In handling the material it has been found necessary (for reasons which will be obvious upon consideration of the matter) to take a fixed date for the beginning of the spring cycle of laying and the ending of the winter cycle. The records of the Station prior to 1908 are tabulated only for months (the daily records unfortunately having been destroyed before I took charge of the work), and on this account it is necessary to take the working limit of the winter cycle at the end of a calendar month. Since March 1 comes the nearest to the biological limit of any date which is also the beginning of a calendar month it has been chosen. The error introduced by taking this arbitrary date for a point which really shifts within rather narrow limits is, on the average, small. However, it must be recognized as a disturbing element in the individual case. Thus, some birds which really lack any genetic factor for winter production will begin to lay in the last days of February, and consequently on the arbitrary 'March 1' basis will actually be credited with a small winter production. This will

high as L_1 would alone. One L_1 cannot, however, build a superstructure on another L_1 ; nor can an L_2 build one on another L_2 . Of course it will be understood that with f (absence of female sex and ovary) these physiological fecundity factors L_1 and L_2 are simply latent.

Using the letters in the manner defined above, and with the usual Mendelian method of writing gametic and zygotic formulæ, the data indicate the existence of Barred Plymouth Rock and Cornish Indian Game males and females of the constitutions set forth below. The only point needing particular attention in reference to these formulæ is that the factor L_2 behaves in inheritance as a sex-limited character precisely like the barred color pattern of the Barred Rock (40, 41). In consequence gametes of the type FL_2 are never formed. Any gamete which bears F does not, under any circumstances, ever carry L_2 .

It is not desirable to take the space to consider here all the consequences which flow from the circumstance of the high fecundity factor L_2 being a sex-limited character. These matters will be fully discussed farther on in the paper after the data themselves have been presented. Here it need only be said that since L_2 is a sex-limited character corresponding in behavior to the barred color pattern, it means that $\sigma\sigma$ may be formed with any combination of the factors L_1 and L_2 , whereas $\phi\phi$ which bear L_2 at all, must be heterozygotic in respect to it. Females may, however, be either homozygotic or heterozygotic in respect to L_1 , it not being a sex-limited character, and hence not in any way coupled with or repelled by the factor F . That the female fowl is heterozygotic in respect to the sex factor was suggested by Spillman (50, 51) and has been demonstrated by the experimental studies of Bateson (3), Goodale (12, 13), Hagedoorn (14), Sturtevant (51) and Pearl and Surface (40, 41).

Tables 5 to 8 inclusive show the constitution in respect to fecundity of males and females of the breeds used in this work, as indicated by the results obtained from breeding experiments. These constitutions represent the 'Erbformeln' which flow from the facts, and, in determination of their adequacy, are to be tested against the facts. In these tables the columns headed

'Gametes produced' have been made up in accord with the general Mendelian principle that in gametogenesis all possible combinations of the factors present will be formed, within the bounds of any limitation which may be imposed by such phenomena as coupling, repulsion or linkage. The limitation of these possibilities in the present instance has been set forth above; it consists simply in the fact that F and L_2 are never borne in the same gamete. It should be said that these tables do not show at all the proportions in which the several gametic types might be expected

TABLE 5

Constitution of Barred Plymouth Rock mules in respect to fecundity

CLASS	ZYGOTE	GAMETES PRODUCED
1	$fL_1L_2 \cdot fL_1L_2$	fL_1L_2
2	$fL_1L_2 \cdot fL_1l_2$	fL_1L_2, fL_1l_2
3	$fL_1L_2 \cdot fl_1L_2$	fL_1L_2, fl_1L_2
4	$fL_1L_2 \cdot fl_1l_2$	$fL_1L_2, fl_1L_2, fl_1l_2, fl_1l_2$
5	$fL_1l_2 \cdot fL_1l_2$	fL_1l_2
6	$fL_1l_2 \cdot fl_1l_2$	fL_1l_2, fl_1l_2
7	$fl_1L_2 \cdot fl_1L_2$	fl_1L_2
8	$fl_1L_2 \cdot fl_1l_2$	fl_1L_2, fl_1l_2
9	$fl_1l_2 \cdot fl_1l_2$	fl_1l_2

TABLE 6

Constitution of Barred Plymouth Rock females in respect to fecundity

CLASS	ZYGOTE	f -BEARING (σ^7 PRODUCING) GAMETES	F -BEARING (σ^7 PRODUCING) GAMETES	PROBABLE WINTER EGG PRODUCTION OF σ^7 OF INDICATED ZYGOTE CONSTITUTION
1	$fL_1L_2 \cdot Fl_1l_2$	$fL_1L_2, fl_1L_2^1$	Fl_1l_2, FL_1l_2	Over 30 eggs
2	$fL_1L_2 \cdot FL_1l_2$	fL_1L_2	FL_1l_2	Over 30 eggs
3	$fL_1l_2 \cdot Fl_1l_2$	fL_1l_2, fl_1l_2	Fl_1l_2, FL_1l_2	Under 30 eggs
4	$fL_1l_2 \cdot FL_1l_2$	fL_1l_2	FL_1l_2	Under 30 eggs
5	$fl_1l_2 \cdot Fl_1l_2$	fl_1l_2	Fl_1l_2	Zero eggs
6	$fl_1L_2 \cdot FL_1l_2$	fl_1L_2	FL_1l_2	Under 30 eggs

¹The reason that gametes of the type fl_1l_2 and fl_1l_2 are not formed here will be evident on consideration. Since no gametes of type FL_2 can, by hypothesis, be formed this implies that an interchange of the factors L_2 and l_2 between F and f gametes cannot occur. The experimental proof of the truth of this conviction has been furnished in the case of the inheritance of the barred color pattern.

to occur. Further all duplicates have been omitted, so that only the different possible types are shown in these tables.

It will be noted from table 6 that two classes of female (1 and 2) carry both L_1 and L_2 and hence are to be expected, the hypothesis developed, to be high layers. One class (class 3) carries neither L_1 nor L_2 and hence should make zero winter records. It should be said that observations indicate that while such class 3 birds occur with expected frequency, they usually do not produce any offspring. A zero winter layer usually gets very few chicks of any kind and almost never has any adult ♀ progeny.

Turning our attention to the Cornish Indian Games, we have the gametic constitutions set forth in tables 7 and 8. The only special point to be noted here is that the factor L_2 does not appear at all in either males or females. All the evidence indicates that in the strain of Cornish Indian Games used in these experiments, this excess production factor L_2 is entirely absent (cf. in this connection tables 1, 2 and 3, supra).

TABLE 7

Constitution of Cornish Indian Game males in respect to fecundity

CLASS	ZYGOTE	GAMETES PRODUCED
1	$fL_1l_2 \cdot fL_1l_2$	fL_1l_2
2	$fL_1l_2 \cdot fl_1l_2$	fL_1l_2, fl_1l_2
3	$fl_1l_2 \cdot fl_1l_2$	fl_1l_2

TABLE 8

Constitution of Cornish Indian Game females in respect to fecundity

CLASS	ZYGOTE	f-BEARING (♂ PRODUCING) GAMETES	F-BEARING (♀ PRODUCING) GAMETES	PROBABLE WINTER EGG PRODUCTION ♀ OF INDICATED ZYGOTIC CONSTITUTION
1	$fL_1l_2 \cdot FL_1l_2$	fL_1l_2	FL_1l_2	Under 30 eggs
2	$fl_1l_2 \cdot FL_1l_2$	fl_1l_2, fL_1l_2	FL_1l_2, Fl_1l_2	Under 30 eggs
3	$fL_1l_2 \cdot FL_1l_2$	fL_1l_2, fl_1l_2	Fl_1l_2, FL_1l_2	Under 30 eggs
4	$fl_1l_2 \cdot FL_1l_2$	fl_1l_2	FL_1l_2	

It will be noted that C.I.G. ♀ classes 2 and 3 are gametically identical. Both are left in the table, however, since the whole table is so short that no confusion can be caused, and this example may make clear to some readers the nature of the compression (by omission of duplicate classes) which was practised in tables 5 and 6.

We may next consider the theoretical results which would be expected to follow the mating in all possible combinations of pairs of the constitutions set forth above. In doing this account will be taken of female progeny *only*, for the sake of simplicity, saving of space, and because we are here concerned only with actual fecundity as expressed in the female. Anyone who desires can easily work out the σ constitutions for himself. Tables 9 and 10 give the expected numbers of female progeny from each mating, on the assumption of uniform fertility throughout. It will be seen that some odd ratios should appear.

It should be pointed out that while, for the sake of completeness, the result of every possible mating is carried out in table 9 on an assumption of equal fertility for all matings, this by no means accords with actual fact. Certain of the matings would not in practice get any offspring at all. This applies also to table 10. This point will be made clear in connection with the application of the theoretical frequencies to the observed data.

It will not be necessary in the table for Cornish Indian Games to present the theoretical frequencies in such detail. Only totals and ratios will be given.

From table 10 it will be seen that no high layers are to be expected from pure Cornish Game matings and that further the proportion of zero layers is relatively high.

ANALYSIS OF THE EXPERIMENTAL DATA

In this section the actual results in respect to fecundity will be compared with the theoretical expectations. There will be presented first the data respecting the matings of Barred Rock males and females (pure B.P.R. matings); second the data respecting matings of Cornish Indian Game males and females (pure C.I.G. matings); and finally the F_1 and F_2 matings of Barred Plymouth Rocks and Cornish Indian Games crossed reciprocally.

Since the actual breeding operations were carried out in advance of any understanding of the mechanism of the inheritance of fecundity the matings were substantially at random so far as con-

TABLE 9

Showing the theoretical expectation in respect to the fecundity of the daughters from all possible matings of Barred Plymouth Rocks inter se

MATINGS		EXPECTED DISTRIBUTION OF FECUNDITY AMONG ♀ PROGENY OF THE DESIGNATED MATING		
B. P. R. ♂ of class	B. P. R. ♀ of class	Daughters with a winter production over 39 eggs	Daughters with a winter production of under 39 eggs	Daughters with a winter production of zero eggs.
1	1 to 6 inclusive	32		
1	All classes	Ratio = 1	0	0
2	1	4	4	
2	2	2	2	
2	3	4	4	
2	4	2	2	
2	5	2	2	
2	6	2	2	
2	All classes	Totals = 16	16	
2	All classes	Ratio = 1	1	0
3	1	6	2	
3	2	4		
3	3	6	2	
3	4	4		
3	5	2	2	
3	6	2	2	
3	All classes	Totals = 24	8	
3	All classes	Ratio = 3	1	0
4	1	3	4	1
4	2	2	2	
4	3	3	4	1
4	4	2	2	
4	5	1	2	1
4	6	1	2	1
4	All classes	Totals = 12	16	4
4	All classes	Ratio = 3	4	1
5	1 to 6 inclusive	22		
5	All classes	Ratio = 0	1	0
6	1		6	2
6	2		4	
6	3		6	2
6	4		4	
6	5		2	2
6	6		2	2
6	All classes	Totals =	24	8
6	All classes	Ratio = 0	3	1

TABLE 9—Continued

MATINGS		EXPECTED DISTRIBUTION OF FECUNDITY AMONG ♀ PROGENY OF THE DESIGNATED MATING		
B. P. R. ♂ of class	B. P. R. ♀ of class	Daughters with a winter production over 30 eggs	Daughters with a winter production of under 30 eggs	Daughters with a winter production of 2 to 10 eggs
7	1	4	1	
7	2	4		
7	3	4	4	
7	4	4		
7	5		4	
7	6		4	
7	All classes	Totals = 16	16	
7	All classes	Ratio = 1	1	0
8	1	2	4	2
8	2	2	2	
8	3	2	4	2
8	4	2	2	
8	5		2	2
8	6		2	2
8	All classes	Totals = 8	16	8
8	All classes	Ratio = 1	2	1
9	1		4	4
9	2		4	
9	3		4	1
9	4		4	
9	5			4
9	6			4
9	All classes	Totals =	16	16
9	All classes	Ratio = 0	1	1
All classes	All classes	Grand Total = 120	160	40
All classes	All classes	Ratio = 3	4	1

concerns fecundity factors. As a consequence not all possible gametic pairings have been made, while for certain combinations a relatively large number of offspring are available. Enough of the possible gametic combinations have, however, been made with Barred Rocks to show clearly how fecundity is inherited.

A word should be said in regard to the number of offspring from the different matings. The writer would, of course, be glad if

TABLE 10

Showing the theoretical expectation in respect to the fecundity of the daughters from all possible matings of Cornish Indian Games inter se

MATING		EXPECTED DISTRIBUTION OF FECUNDITY AMONG PROGENY OF THE DESIGNATED MATING		
C. I. G. ♂ of class	C. I. G. ♀ of class	Daughters with a winter record of over 30 eggs	Daughters with a winter record of under 30 eggs	Daughters with a winter record of zero eggs
1	1 to 4 inclusive.		12	
1	All classes	Ratio = 0	1	0
2	1 to 4 inclusive	Totals	9	3
2	All classes	Ratio = 0	3	1
3	1 to 4 inclusive	Totals	6	6
3	All classes	Ratio = 0	1	1
All classes	All classes	Grand totals	27	9
		Ratio = 0	3	1

records were at hand for a large number of progeny for every mating made. There are, however, practical difficulties in the matter. The Maine Experiment Station poultry plant has accommodations for only about 600 adult pullets per annum in spite of the fact that it is one of the largest purely experimental poultry plants in the country.⁹ Now taking all the experiments together there are made about 300 separate matings each year. It is simple arithmetic to show that under the circumstances, if all matings were equally represented, only two pullets from each mating could be tested as to fecundity. As a matter of fact all matings are not equally represented. Some yield either no chickens, or too few to insure the development of adult daughters. The aim has always been in this work to put into the laying house for trap-nest records of fecundity as many daughters from each one of as many matings as possible. Of course, only healthy, normal

⁹ There are hatched annually on this plant from 3500 to 4000 chicks, and facilities for handling adult stock make it possible to accommodate over winter about 1000 birds of all sorts, including adult pullets, hens and male birds.

well developed pullets can be used in the work, since any other sort could not be depended upon to give reliable normal results as to fecundity. This means that, under the prevailing climatic conditions here, only pullets hatched between a rather narrow range of dates (April 1 to June 1) can be used in the fecundity. Those hatched at other seasons will not give normal results.

Altogether it will be seen that the character fecundity in fowls is not one which lends itself readily to treatment in large masses of figures, desirable as such might theoretically be. The case is very different from the study of the inheritance of plumage colors in poultry, for example, where both sexes are available for record and the records may be made while the chicks are relatively young (or in some cases even unhatched) and before they have time to die. If all students of the inheritance of pigmentation in poultry had been obliged to keep, house, and feed every bird which was to furnish any record whatever, until approximately one and a half years after hatching, and could have got records even then only from one sex (both of which conditions obtain in the study of fecundity), it is plain that their recorded numbers would have fallen very far below those which they have actually, and most fortunately for the good of biology, been able to obtain.

The foregoing remarks are not in any sense intended as an apologia for the statistical portion of this paper, because in the opinion of the writer, who is thoroughly acquainted with the practical difficulties which beset the study of inheritance of fecundity, no apology is needed. The data here presented are about as extensive as it is practically possible to obtain in an interval of time and with an experimental equipment equal to what has been available in the present investigation. It is hoped, however, that what has been said may help the reader, who may not be practically familiar with the rearing and trap-nesting of large numbers of fowls, to understand the reason why more extensive data are not forthcoming in this paper. In every case where the number of birds to a family was too small to warrant any conclusion this fact is particularly noted. The data for these small families are not suppressed, however, but are in most instances separately tabulated.

One convention which is used throughout in the tabulation of the material should be explained. In case a bird has a winter egg record of exactly 30 eggs, she evidently falls on the boundary line between the two fecundity classes already discussed and defined (p. 172). The number of such cases is not large, but in order to be perfectly impartial in their treatment it was decided to split such a bird in two, in a metaphorical sense, and credit one-half of her to the 'Over 30' winter fecundity class, and the other half to the 'Under 30' class. This explains the fractional records which occasionally appear among the frequencies in what follows, and which might otherwise puzzle one used to thinking of a hen as an individual unit, at least during the fecund portion of her existence. In calculating the mean winter production (in eggs) of the several classes these few birds with records of exactly 30 eggs have been omitted altogether. There are obviously two equally fair ways of dealing with them in getting these averages. One is to include each one in *both* 'over' and 'under' classes; the other is to include each one in *neither* class. The latter alternative is adopted because simpler.

Barred Plymouth Rock matings

The data will be presented for each gametic constitution separately. The analysis indicates that out of the 9 theoretically possible types of male Barred Rocks shown in table 5 only six have actually ever been used in the breeding pens. These six classes of males represented in the data are classes 1, 2, 3, 4, 7 and 8.

In any particular case it is practicable to determine the gametic constitution of a male bird in respect to fecundity only through an examination of the records of his daughters. To distinguish different gametic types of males through analysis of the male progeny, while theoretically simply, is practically not feasible while any other investigations are going on. In order to determine the gametic constitution in regard to fecundity of the cockerels from a particular mating it would be necessary to rear to maturity a reasonable number (5 to 10) of these males, and

then a year from the time they were hatched to mate each of them with a number of females, and rear to maturity and trap-nest for a year a number (3 to 10 for example) of pullets grown from each of the matings of each of the cockerels. Then from the trap-nest records of these pullets it would be possible to conclude as to what was their grandfather's gametic constitution respecting fecundity. It is evident that relatively enormous experimental resources would be required to carry this out on even a very modest scale. Further the end would scarcely justify the means from either a practical or theoretical standpoint, since the theoretically expected gametic types of males can be readily obtained and their pedigrees will enable one to analyze fully the gametic factors and reactions involved in their production.

Throughout the paper, then, conclusions will be drawn as to gametic constitution of parents from an analysis of the female progeny only.

The reason why the other three classes of males (5, 6 and 9) are not represented in the matings is to be found in the method of selective breeding practised during the time in which the statistics here analyzed were collected. The chance of using in a breeding pen males of any of these types was small when the selection was carried on in the way that it was. This point will be more fully discussed farther on in the paper.

Matings of Barred Plymouth Rock males of class¹⁰ 7

Males of class 7, having a gametic constitution $fl_1L_2 \cdot fl_1L_2$, were used more often than any other sort in the pure Barred Rock matings. They are homozygous with reference to the absence of the first production factor L_1 , and the presence of the second or excess production factor L_2 . A reference to table 9 shows that there should be no zero winter producers among their progeny. The proportions of high and poor layers in the progeny depend upon the nature of the female with which the male is bred. For convenience the matings of each individual male will be discussed separately.

¹⁰ The 'class' numbers throughout refer to the arbitrary designations given in tables 5 to 10 inclusive.

B.P.R. ♂ 553. Indicated gametic constitution = $fl_1L_2 \cdot fl_1L_2$.

This male was hatched in the spring of 1908 and used as a breeder in the season of 1909. His successful 'pure' matings (i.e., those with B.P.R. females which produced adult female progeny) were as follows:

Matings: A. With 6 ♀♀ indicated to be of class 2 = $fL_1L_2 \cdot fL_1l_2$.

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
<i>Observed</i>	15	0	0
<i>Expected</i>	15	0	0
Mean winter production of ♀♀ in indicated class.....	51.33 eggs		

B. With 3 ♀♀ indicated to be of class 1 = $fL_1L_1 \cdot FL_1l_2$.

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
<i>Observed</i>	6	9	0
<i>Expected</i>	7.5	7.5	0
Mean winter production of ♀♀ in indicated class.....	55.50 eggs	13.56 eggs	

C. With 2 ♀♀ indicated to be of class 4 = $fl_1l_2 \cdot FL_1l_2$.

♀ Progeny			
<i>Winter Production</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
<i>Observed</i>	4	0	0
<i>Expected</i>	4	0	0
Mean winter production of ♀♀ in indicated class.....	39.75 eggs		

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
<i>Observed</i>	25	9	0
<i>Expected</i>	26.5	7.5	0
Mean winter production.....	50.48 eggs	13.56 eggs	

The agreement between observation and expectation here is as close as could be expected considering the numbers involved. Further it is evident from the mean production of the daughters falling in the several classes that the 'Over 30' and 'Under 30' classes are perfectly distinct in respect to degree of fecundity.

The 'Over 30' birds produced on the average, nearly four times as many eggs in the winter period as the 'Under 30' birds.

B.P.R. ♂ 567. Indicated gametic constitution = $fl_1L_2 \cdot fl_1L_2$.

This male was used in the breeding season of 1910 and sired a fairly large number of birds of which the adult daughters appear below. He was hatched in the spring of 1909.

Matings: A. With 5 ♀♀ indicated to be of class 1 = $fl_1L_2 \cdot Fl_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	7½	9½	0
Expected.....	8.5	8.5	0
Mean winter production of ♀♀ in indicated class.....	57.28 eggs	14.78 eggs	

B. With 3 ♀♀ indicated to be of class 2 = $fl_1L_2 \cdot Fl_1l_2$

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	12	2	0
Expected.....	14	0	0
Mean winter production of ♀♀ in indicated class.....	55.83 eggs	22.00 eggs	

C. With 2 ♀♀ indicated to be of class 6 = $fl_1L_2 \cdot Fl_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	1½	3½	0
Expected.....	0	5	0
Mean winter production of ♀♀ in indicated class.....	48.00 eggs	14.00 eggs	

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	21	15	0
Expected.....	22.5	19.5	0
Mean winter production.....	55.95 eggs	15.64 eggs	

It will be noted that there are two exceptions in these matings. A class 7 ♂ × class 2 ♀ should give only daughters in the 'Over 30' class. Two out of the 14 adult progeny from matings of this type laid fewer than 30 eggs in the winter period. The record of

one of these two was 28 eggs. There is no doubt that this bird was a somatic variation belonging gametically to the 'Over 30' class. (cf. p. 173). In general it is obvious that the agreement between observation and expectation here is very satisfactory. Further the difference in average winter production of the birds in the 'Over 30' and 'Under 30' classes is so great as to leave no doubt of the real distinctness of these classes in respect to fecundity.

B.P.R. 562. Indicated gametic constitution = $fl_1L_2 : fl_1L_2$.

This male got comparatively few adult daughters. He was used during only one breeding season (that of 1910), having been hatched in the spring of 1909.

Matings: A. With 4 ♀♀ indicated to be of class 1 = $fl_1L_2 : fl_1L_2$.

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	5	6	0
Expected.....	5.5	5.5	0
Mean winter production of ♀♀ of indicated class.....	42.40 eggs	11.67 eggs	

B. With 2♀♀ indicated to be of class 2 = $fl_1L_2 : fl_1L_2$.

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	8	0	0
Expected.....	8	0	0
Mean winter production of ♀♀ of indicated class.....	70.00 eggs		

<i>All ♀ Progeny</i>			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	13	6	0
Expected.....	13.5	5.5	0
Mean winter production.....	59.38 eggs	11.67 eggs	

In spite of the comparatively small number of individuals here, the evidence of segregation of high and low fecundity in accordance with gametic expectation is clear and indubitable.

B.P.R. ♂ 552. Indicated gametic constitution = $fl_1L_2 \cdot fl_1L_2$.

This male was used as a breeder during two seasons (1909 and 1910). He was hatched in the spring of 1908. His sisters were very poor winter layers, as shown by the following table.

Sisters of ♂ 552	Winter production as pullets
	Eggs
E 184	16
E 229	6
E 272	7
Mean winter production of family.....	9.67

The mother of ♂ 552 (♀ D725) was a good layer with a winter record of 61 eggs. From her he evidently got an L_2 factor which his sisters could not acquire in this way. The father was heterozygous relative to L_2 (belonging to class 4) and the only one of his adult progeny from the mating with ♀ D725 to bear L_2 happened to be the ♂ 552 here under discussion. In the following account of ♂ 552's breeding history the progeny in both of the years in which he was used in the pens are taken together. There is no reason why the two years should be dealt with separately.

Matings: A. With 4 ♀ ♀ indicated to be of class 2 = $fl_1L_2 \cdot Fl_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	11½	½	0
Expected.....	12	0	0
Mean winter production of ♀ ♀ in indicated class.....	48.27 eggs		

B. With 10 ♀ ♀ indicated to be of class 1 = $fl_1l_2 \cdot Fl_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	17	15	2
Expected.....	17	17	0
Mean winter production of ♀ ♀ in indicated class.....	54.71 eggs	12.47 eggs	0 eggs

C. With 3 ♀♀ indicated to be of class 3 = $fl_1l_2 \cdot Fl_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	2	1	0
Expected.....	1.5	1.5	0
Mean winter production of ♀♀ in indicated class.....	35.50 eggs	22.00 eggs	

All ♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	30½	16½	2
Expected.....	30.5	18.5	0
Mean winter production.....	51.07 eggs	13.06 eggs	0

In this case the two zero birds are without much question to be reckoned as somatic rather than genetic zeros. Unfortunately neither of these birds were bred, so that precise information on the point is lacking. Assuming this to be the case the agreement between observation and expectation in the large progeny is perfect. The matings under C got so few ♀ progeny as to be without significance one way or the other.

The mean winter productions again show the distinctness of the separation between the 'Over 30' and 'Under 30' fecundity classes.

B.P.R. ♂ 554. Indicated constitution = $fl_1L_2 \cdot fl_1L_2$.

This bird, like ♂ 552 was used in the breeding pens two years. He was hatched in 1908 and bred in each of the two following years. His breeding history was as follows:

Matings: A. With 8 ♀♀ indicated to be of class 1 = $fl_1L_2 \cdot Fl_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	12	12	1
Expected.....	12.5	12.5	0
Mean winter production of ♀♀ in indicated class.....	47.67 eggs	15.58 eggs	0 eggs

B. With 3 ♀♀ indicated to be of class 2 = f_1l_2 . F_1l_2 .

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	8	0	0
Expected.....	8	0	0
Mean winter production of ♀♀ in indicated class.....	59.00 eggs		

C. With 2 ♀♀ indicated to be of class 6 = $f_1l_1l_2$. $F_1l_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	0	3	0
Expected.....	0	3	0
Mean winter production of ♀♀ in indicated class.....	20.33 eggs		

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	20	15	1
Expected.....	20.5	15.5	0
Mean winter production.....	32.20 eggs	16.53 eggs	0 eggs

Barring the single bird with a zero record the agreement between observation and expectation here is perfect. This exception was a late¹¹ hatched bird (June 2, 1910). It laid an egg on May 1, 1911, of its pullet year, and died from a combination of pulmonary and intestinal difficulties on May 22. Under these circumstances it obviously carries little weight as an exception to expectation on a gametic basis.

B.P.R. ♂ 564. Indicated constitution = $f_1l_1l_2$. $f_1l_1l_2$.

This bird was hatched in 1909 and used in the breeding season of 1910, with the following results:

¹¹ It must always be remembered that 'late' is relative. Under our conditions of climate, etc., at Orono, June 2 represents very late hatching for birds which are to be used in fecundity work. The cold weather comes on so early in the fall and is so severe that any bird not fully developed by the middle of October or the first of November at the latest is likely to remain permanently stunted. The first of June represents about the latest possible limit of hatching for fecundity work under these conditions.

Matings: A. With 6 ♀♀ indicated to be of class 1 = $fL_1L_2 \cdot Fl_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	9	10	2
<i>Expected.....</i>	<i>10.5</i>	<i>10.5</i>	<i>0</i>
Mean winter production of ♀♀ in indicated class.....	64.44 eggs	19.80 eggs	0 eggs

B. With 3 ♀♀ indicated to be of class 3 = $L_1l_2 \cdot Fl_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	2	2	0
<i>Expected.....</i>	<i>2</i>	<i>2</i>	<i>0</i>
Mean winter production of ♀♀ in indicated class.....	54.50 eggs	26.50 eggs	

C. With 1 ♀ indicated to be of class 6 = $fL_1L_2 \cdot Fl_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	0	2	0
<i>Expected.....</i>	<i>0</i>	<i>2</i>	<i>0</i>
Mean winter production of ♀♀ in indicated class.....		4.00 eggs	

	All ♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	11	14	2
<i>Expected.....</i>	<i>12.5</i>	<i>14.5</i>	<i>0</i>
Mean winter production.....	62.64 eggs	18.85 eggs	0 eggs

The families are small in this case. From both these pure Barred Rock and the cross matings in which ♂ 564 entered, however, there can be no doubt that he is a class 7 male. The two zero birds are to be reckoned as 'somatic zeros' rather than gametic. Both began laying at the very beginning of the spring period, and made records which indicated to one familiar with this sort of material that they belonged genetically in the 'Under 30' class and only by accident failed to lay some eggs during the winter period.

B.P.R. ♂ D53. Indicated constitution = $f l_1 L_2 \cdot f l_1 L_2$.

This bird was purchased in January, 1908, from Gardner & Dunning, a then well-known firm of Barred Rock breeders of Auburn, N. Y. . Nothing was known of this bird's previous history or pedigree. The bird was hatched in the spring of 1907, and used in our breeding pens in 1908 and 1909. In 1908 he failed to get any adult daughters. This, however, was not the fault of the bird, but of the conditions under which the breeding had to be done that year (cf. Pearl and Surface 35). From the records of the daughters of ♂ 58 obtained in 1909 and exhibited below it appears clear that he was a class 7 male. The breeding history is as follows:

Matings: A. With 9 ♀ ♀ indicated to be of class 1 = $f L_1 L_2 \cdot F l_1 l_2$.

♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	10	13	1
Expected.....	12	12	0
Mean winter egg production of ♀ ♀ in indicated class.....	52.22 eggs	17.25 eggs	0 eggs

B. With 4 ♀ ♀ indicated to be of class 6 = $f l_1 L_2 \cdot F l_1 l_2$.

♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	0	5	0
Expected.....	0	5	0
Mean winter egg production of ♀ ♀ in indicated class.....		15.80 eggs	

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	10	18	1
Expected.....	12	17	0
Mean winter production	52.22 eggs	16.82 eggs	0 eggs

The single zero bird here (♀ F158) cannot fairly be regarded as a non-conformable case because of the following history. She was hatched March 30, 1909. She never laid an egg and died May 23, 1910. Autopsy showed the ovary and oviduct to be in an infantile condition. The ovary weighed 1 gram and the oviduct

2 grams. The ovary showed no oöcytes enlarged by yolk deposition or enlarging. There was no evidence that the ovary had ever shown the slightest trace of functional activity. But a normal bird hatched in March will exhibit signs of ovarian activity before May of the following year, even though she belongs genetically to the 'Zero' class in respect to winter production and does not lay. While the autopsy showed no obvious lesion of ovary or oviduct, this by no means proves that there may not have been present some deep-seated functional derangement.

B.P.R. ♂ 573. Indicated constitution = $fL_1L_2 \cdot fL_1L_2$.

This bird was used in the breeding season of 1910, having been hatched in 1909. He proved not to be all that might be desired as a breeder, being somewhat lacking in vigor of constitution. Partly on this account, he got comparatively few adult daughters, as indicated in the following breeding history.

Matings: A. With 5 ♀ ♀ indicated to be of class 1 = $fL_1L_2 \cdot fL_1L_2$.

	♀ Progeny		
Winter Production:	Over 30	Under 30	Zero
Observed.....	4½	6½	0
Expected.....	5.5	5.5	0
Mean winter production of ♀ ♀ in indicated class.....	47.50 eggs	15.67 eggs	

B. With 2 ♀ ♀ indicated to be of class 2 = $fL_1L_2 \cdot fL_1L_2$.

	♀ Progeny		
Winter Production:	Over 30	Under 30	Zero
Observed.....	4	0	0
Expected.....	4	0	0
Mean winter production of ♀ ♀ in indicated class.....	49.25 eggs		

C. With 1 ♀ indicated to be of class 3 = $fL_1l_4 \cdot fL_1l_2$.

	♀ Progeny		
Winter Production:	Over 30	Under 30	Zero
Observed.....	2	1	1
Expected.....	2	2	0
Mean winter production of ♀ ♀ in indicated class.....	55.50 eggs	16.00 eggs	0 eggs

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
<i>Observed</i>	10½	7½	1
<i>Expected</i>	11.5	7.5	0
Mean winter production.....	49.80 eggs	15.71 eggs	0 eggs

The zero bird here is an exception for which no apparent explanation is forthcoming. She was not pathological. She was however a June hatched bird. Unfortunately she was not bred, and therefore it is not possible to be sure of her gametic constitution. In spite of the fact that the total number of progeny here is small, there is little doubt of the correctness of the classification.

The mean productions for birds in the 'Over 30' class in the several matings are comparatively a little lower than those of the progeny of other class 7 males. It is interesting to speculate as to whether this may be connected with the lack of great vigor on the part of the sire. No data are available from which to get critical evidence on this point.

B.P.R. ♂ 56. Indicated constitution = $fl_1L_2 \cdot fl_1L_2$.

This bird was purchased in January, 1908, from Mr. C. H. Welles of Stratford, Conn. It came from a strain of Barred Rocks well known in the show-room, but not specially bred for egg production. This fact is of interest in connection with the breeding history of the bird, which indicates clearly that he was homozygous with respect to L_2 . The result shows, in other words, that a male Barred Rock from a strain bred purely for the fancy may still carry in pure form the factor for high egg production.

This male bird (56) was bred two seasons (1908 and 1909). The first year he got but very few adult daughters, owing to the unfavorable conditions under which all the breeding had to be done in 1908 (cf. Pearl and Surface 35). In 1909 the results were better. The adult daughters from both seasons are taken together in the following breeding history.

Matings: A. With 5 ♀♀ indicated to be of class 2 = $fL_1L_2 \cdot FL_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	7	0	0
Expected.....	7	0	0
Mean winter production of ♀♀ in indicated class.....	51.57 eggs		

B. With 4 ♀♀ indicated to be of class 3 = $fL_1l_2 \cdot Fl_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	9	4	0
Expected.....	6.5	6.5	0
Mean winter production of ♀♀ in indicated class.....	56.89 eggs	19.50 eggs	

C. With 2 ♀♀ indicated to be of class 6 = $fl_1L_2 \cdot Fl_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	0	3	0
Expected.....	0	3	0
Mean winter production of ♀♀ in indicated class.....		13.67 eggs	

	<i>All ♀ Progeny</i>		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	16	7	0
Expected.....	13.5	9.5	0
Mean winter production.....	55.87 eggs	17.00 eggs	0

The agreement between observation and expectation here is satisfactory, excepting the case of the class 3 females. There the deviation from the expected half is wide, but the numbers involved are small. The behavior of ♂ 56 with class 2 and class 6 females gives clear indication of his gametic constitution.

B.P.R. ♂ 563. Indicated constitution = $fl_1L_2 \cdot fl_1L_2$.

This bird was hatched in 1909 and used as a breeder in 1910. He was an exceptionally fine, vigorous bird. The breeding history is as follows:

Matings: A. With 6 ♀♀ indicated to be of class 1 = fL_1L_2 . FL_1L_2 .

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	11	11	1
Expected.....	11.5	11.5	0
Mean winter production of ♀♀ in indicated class.....	64.09 eggs	17.91 eggs	0 eggs

B. With 5 ♀♀ indicated to be of class 2 = fL_1L_2 . FL_1L_2 .

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	18	1	0
Expected.....	19	0	0
Mean winter production of ♀♀ in indicated class.....	63.56 eggs	1.00 eggs	

All ♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	29	12	1
Expected.....	30.5	11.5	0
Mean winter production.....	63.76 eggs	16.50 eggs	0 eggs

Aside from the two outstanding exceptions the agreement between observation and expectation is excellent. From the records available there is no evident explanation for the two exceptions (the 'Zero' bird in the A matings, and the 'Under 30' bird in the B matings). Neither of the birds were bred, and hence no help is to be had from the progeny in explaining them. It is reasonable to suppose that the observed records for these birds are somatic fluctuations, but this cannot be demonstrated now. This case illustrates an unavoidable difficulty which attends that method of work which first collects data at random and without any theoretical guide, and then later undertakes their analysis. If one had been carrying on the breeding in the present case under the guidance of the hypothesis as to the mechanism of the inheritance of fecundity now under discussion, obviously many matings which actually were not carried out would have been made to test out somatically exceptional individuals and so learn their gametic constitution.

B.P.R. ♂ D31. Indicated constitution = $fl_1L_2 \cdot fl_1L_2$.

This rather remarkable bird was used as a breeder for three successive years, and then retired merely because no more of his progeny were needed, and not for any evident diminution of vigor on his part. This bird was first bred as a cockerel in the spring of 1908 (hatched in 1907). All that was known of his ancestry was that he was the son of a hen that had laid 200 or more eggs in her pullet year. Some notion of the vigor of *c* D31 as a breeder may be gained from the fact that, taking all three seasons together and including all parts of the breeding season in each year, 89.4 per cent of all the eggs laid by hens mated with him were fertile. This is an extraordinarily high record, considering all the circumstances, and particularly the seasonal and housing conditions. So far as concerns adult daughters the breeding history of this bird is as follows:

Matings: A. With 9 ♀ ♀ indicated to be of class 1 = $fl_1L_2 \cdot fl_1L_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	10½	11½	0
Expected.....	11	11	0
Mean winter production of ♀ ♀ in indicated class.....	48.40 eggs	15.73 eggs	

B. With 8 ♀ ♀ indicated to be of class 2 = $fl_1L_2 \cdot FL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	27½	2½	0
Expected.....	30	0	0
Mean winter production of ♀ ♀ in indicated class.....	54.96 eggs	17.00 eggs	

C. With 8 ♀ ♀ indicated to be of class 3 = $fl_1l_2 \cdot Fl_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	14	15	1
Expected.....	15	15	0
Mean winter production of ♀ ♀ in indicated class.....	41.93 eggs	12.20 eggs	0 eggs

D. •With 1 ♀ indicated to be of class 4 = fL_1l_2 . FL_1l_2 .

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	5	0	1
Expected.....	6	0	0
Mean winter production of ♀♀ in indicated class.....	39.40 eggs		0 eggs

•All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	57	29	2
Expected.....	62	26	0
Mean winter production.....	48.16 eggs	13.81 eggs	0 eggs

Besides the families noted above ♂ D31 got one adult daughter by each of two other females. Both these daughters had a winter record of zero eggs and were apparently pathological. In any event it was impossible to form any judgment as to their gametic constitution or that of their dams.

The general agreement between observation and expectation in this large progeny group is clear. The apparent exceptions to gametic expectation need some discussion. In the B matings (class 2 ♀ ♀) the record shows $2\frac{1}{2}$ in the 'Under 30' class where none is expected. Actually out of the 30 individuals from these matings only *one* daughter laid fewer than 30 eggs in the winter period. There were, however, 3 individuals which laid exactly 30 eggs in this period. So, in accordance with the convention adopted at the beginning, the record of $2\frac{1}{2}$ is made up as follows: $1 + \frac{1}{2} + \frac{1}{2} + \frac{1}{2} = 2\frac{1}{2}$. The one bird under 30 with a record of 17 eggs was late hatched and probably represented a somatic fluctuation. This bird was bred, but unfortunately got no offspring. Her eggs were nearly all fertile but the embryos died during incubation.

Of the two birds with a zero winter record it may be said that one (E96) was pathological, and on that account failed to lay. The autopsy on this bird, which died April 13, 1909, showed that it must have been functionally deranged for a long time preceding death. Yet there was clear evidence of functional activation of ovary and oviduct at some time before death. In this case the

bird without question carried either L_1 or L_2 (or possibly both) and the reproductive system started to function in the normal way and bring to somatic expression these gametic factors. But before this could be done the diseased condition of the organs brought the bird as a whole into such a condition of reduced vitality that egg production was impossible.

The other bird's zero record is probably a somatic fluctuation from an 'Under 30' hereditary constitution. She began laying very shortly after the end of the winter period.

It is of interest to note that the mean winter productions are relatively rather low for the 'Over 30' classes in all matings. The contrast between ♂ D31 progeny and that of ♂ 563 (vide supra) in this respect is striking. This matter will be discussed in detail later.

Summary and discussion of matings of class 7 Barred Plymouth Rock males. Having now presented in detail the evidence respecting the matings of class 7 males with various types of females it is desirable to collect and summarize this material. In tables 11 to 16 inclusive are given the assembled results of all matings of certain particular types. It will be understood that these are all pure Barred Rock matings and represent the summation of the data previously given. These tables give the total numbers of different males and females from which data were obtained in each class of matings, as well as the classification of the adult female progeny in respect to fecundity.

TABLE 11
Showing the results of all matings of class 7 ♂ ♂ × class 1 ♀ ♀
 $f l_1 L_1 : f l_1 L_2 \times f L_1 L_2 : F l_1 l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF ADULT DAUGHTERS				
♂ ♂	♀ ♀	Class	Over 30	Under 30	Zero	Total adult offspring
10	75	Observed	92½	103½	7	203
		Expected	101.5	101.5	0	

Mean winter egg production of all daughters in designated

class..... 51.19 eggs 15.52 eggs 0 eggs

TABLE 12

Showing the results of all matings of class 7 ♂♂ × class 2 ♀♀
 $fl_1L_2 \cdot fl_1L_2 \times fl_1L_2 \cdot FL_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF ADULT DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult ♀ offspring
9	38	Observed	111	6	0	117
		Expected	117	0	0	

Mean winter egg production of all daughters in designated class 56.47 eggs 20.33 eggs

TABLE 13

Showing the results of all matings of class 7 ♂♂ × Class 3 ♀♀
 $fl_1L_2 \cdot fl_1L_2 \times fl_1L_2 \cdot FL_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF ADULT DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult ♀ offspring
5	19	Observed	29	23	2	54
		Expected	27	27	0	

Mean winter production of all daughters in designated class 47.93 eggs 15.30 eggs 0 eggs

TABLE 14

Showing the results of all matings of Class 7 ♂♂ × Class 4 ♀
 $fl_1L_2 \cdot fl_1L_2 \times fl_1L_2 \cdot FL_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF ADULT DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult ♀ offspring
2	3	Observed	9	0	1	10
		Expected	10	0	0	

Mean winter egg production of all daughters in designated class 39.56 eggs 0 eggs

TABLE 15

Showing the results of all matings of class 7 ♂♂ × class 6 ♀♀
 $fl_1L_2 \cdot fl_1L_2 \times fl_1L_2 \cdot Fl_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF ADULT DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total Adult offspring
5	11	Observed	1½	16½	0	18
		Expected	0	18	0	

Mean winter egg production of
 all daughters in designated
 class 48.00 eggs¹ 14.44 eggs

¹ The record of the single 'Over 30' bird.

TABLE 16

Showing the results of all matings of class 7 ♂♂ with all classes of ♀♀
 General Summary

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF ADULT DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult offspring
11	146	Observed	243.0	149.0	10	402
		Expected	255.5	146.5	0	

Mean winter egg production of
 all daughters in designated
 class 53.67 eggs 15.37 eggs 0 eggs

From these tables the following points would appear to be definitely established:

1. The numbers of different individuals used as parents in these matings and also the numbers of adult daughters obtained from them are great enough to give an adequate test of the hypothesis under discussion. In other words, we are not dealing here with the results of a few matings, and a small offspring series. One hundred and forty-six separate and distinct matings to test out males of one gametic constitution must be regarded as an adequate number.

2. *The evidence for a definite and clean-cut segregation of high fecundity and low fecundity in gametogenesis is clear and indubitable.* The expected proportions of high producers and low producers are closely realized in all the different types of matings.

3. Furthermore, the mean egg productions of the birds in the several gametic classes are widely separated, showing that the segregation is of perfectly distinct physiological entities. Refined biometric tests are not necessary to show that the birds carrying high fecundity hereditarily lay more than those with low fecundity hereditary factors. The birds in the 'Over 30' class have average winter productions from three to five times greater than those of birds belonging to the 'Under 30' class.

4. The agreement between observation and expectation for the several types of mating is as close as could be expected considering the nature of the material. The only discrepancy of note is caused by the 10 birds with zero records, where none are expected. In the detailed discussions in connection with each mating it has been shown, however, that nearly all of these 10 cases, when studied individually, have a physiological explanation, which makes it impossible to regard them as real exceptions to the gametic expectations. A determination might be made of the 'goodness of fit' of theory to observation by Pearson's (42) method, were it not for the fact that that method cannot be applied to cases like the present.¹²

¹² The difficulty lies in the fact that Pearson's test depends upon a variable

$$\chi^2 - S \left\{ \frac{(m_r - m'_r)^2}{m_r} \right\}$$

where m_r is the theoretical frequency and m'_r the observed. Now obviously in any distribution where even one m_r is zero, the value of χ^2 must be infinity, whatever may be the values of the other m_r 's or m'_r 's. That is, if the theoretically expected frequency on any base element is numerically zero the probability against the whole curve becomes infinite. Thus, for example, suppose a system of frequencies like the following, a type which is continually arising in Mendelian work.

Class.....	1	2	3	4	5
Theoretically expected frequency...	505	827	68	0	96
Actually observed frequency.....	594	828	67	1	96

Now, it does not need a mathematical measure of any kind to tell one that in this case the theoretical and actual distributions are in very close agreement.

Further discussion of various points brought out by these tables is deferred to a later section of the paper.

Matings of Barred Plymouth Rock males of class 4.

Males of class 4 have a gametic constitution $fL_1L_2 \cdot fl_1l_2$. That is, they are heterozygous with respect to both fecundity factors. Among the progeny are to be expected high, low and zero winter layers. Four male birds of this genotypic constitution have been used in the breeding experiments. Their records follow.

B.P.R. ♂ 569. Indicated constitution = $fL_1L_2 \cdot fl_1l_2$.

This male was hatched in 1909, and bred the following year. His breeding history was as follows:

Matings: A. With 1 ♀ indicated to be of class 2 = $fL_1L_2 \cdot FL_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	2	0	0
Expected.....	1	1	0
Mean winter egg production of ♀ ♀ indicated class.....	67.00 eggs		

Yet, because the *theoretical* frequency on class 4 is zero, the probability by Pearson's test is literally infinite *against* the observed distribution being regarded as a random sample of a population distributed in accordance with the theoretical frequencies. Pearson (loc. cit., p. 164, footnote) had indeed himself noted what is essentially this same difficulty in using the test on ordinary frequency distributions.

The point noted obviously limits greatly the applicability of Pearson's test, and in a most unfortunate direction. Tests of goodness of fit are much needed in Mendelian work. But it is just here that classes where the theoretical frequency is zero often occur. To determine the probable error of the individual frequency in measuring the goodness of fit of Mendelian observation and theory, as was first practised by Weldon (52) and later by Johannsen (21) and by Mendelian workers generally, does not appear to the writer to be an altogether sound procedure. It fails to take account of the *correlations* in errors amongst the several frequencies. Yet these are just as important and just as certainly existent in a Mendelian 'category' type of distribution as in the ordinary variation polygon of a continuously variable character. This point I have alluded to elsewhere recently (Pearl, 32). Pearson's test covers this point, and were it not for the other difficulty noted above would be much more widely useful in Mendelian work than is actually the case.

B. With 4 ♀♀ indicated to be of class 0 = $f l_1 l_2 \cdot F l_1 l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	2	6	3
Expected.....	2.75	5.5	2.75
Mean winter production of ♀♀ in indicated class.....	75.00 eggs	7.33 eggs	0 eggs

C. With 4 ♀♀ indicated to be of class 1 = $f L_1 L_2 \cdot F l_1 l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	5½	6½	1
Expected.....	4.9	6.5	1.6
Mean winter production of ♀♀ in indicated class.....	44.60 eggs	8.00 eggs	0 eggs

D. With 3 ♀♀ indicated to be of class 4 = $f L_1 l_2 \cdot F L_1 l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	3	3	0
Expected.....	3	3	0
Mean winter egg production of ♀♀ in indicated class.....	45.33 eggs	7.33 eggs	0 eggs

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	12½	15½	4
Expected.....	11.65	16	4.35
Mean winter production.....	53.58 eggs	7.00 eggs	0 eggs

The agreement between observation and expectation is plainly very close here. The three fecundity classes are represented and in proportions as near to those indicated by hypothesis as could be expected, considering the numbers involved.

B.P.R. ♂ 566. Indicated constitution = $f L_1 L_2 \cdot f l_1 l_2$.

This bird was used in the breeding pen in the season of 1910, having been hatched in the spring of the previous year. His sire was ♂ D556, a class 4 male to be taken up later, and his dam a class 2 female. His breeding history was as follows:

Matings: A. With 5 ♀♀ indicated to be of class 1 = $fL_1L_2 \cdot Fl_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	4	8	2
<i>Expected</i>	5.2	7	1.8
Mean winter egg production of ♀♀ in indicated class.....	35.00 eggs	20.50 eggs	0 eggs

B. With 6 ♀♀ indicated to be of class 2 = $fL_1L_2 \cdot FL_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	9	6	0
<i>Expected</i>	7.5	7.5	0
Mean winter eggs production of ♀♀ in indicated class.....	50.44 eggs	11.83 eggs	0 eggs

	<i>All ♀ Progeny</i>		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	13	14	2
<i>Expected</i>	12.7	14.5	1.8
Mean winter production.....	45.69 eggs	16.79 eggs	0 eggs

Here again the agreement between observation and expectation is very close, quite as close as could be expected with the numbers involved. The mean production of the 4 birds in the 'Over 30' class in the A matings is low.

B.P.R. ♂ D35. Indicated constitution = $fL_1L_2 \cdot fl_1l_2$.

This bird was one of the original males with which the present breeding experiments were started in 1908. The only thing known about his ancestry is that he was the son of a hen laying 200 or more eggs in the year. He got only a small adult female progeny, and was used as a breeder only one year. His breeding record follows.

Matings: A. With 4 ♀♀ indicated to be of class 1 = $fL_1L_2 \cdot Fl_1l_2$.

	♀ Progeny		
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	3	5	1
<i>Expected</i>	3.4	4.5	1.1
Mean winter production of ♀♀ in indicated class.....	58.67 eggs	15.20 eggs	0 eggs

E. With 2 ♀♀ indicated to be of class 3 = $fL_1l_2 \cdot FL_1l_2$:

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	2	5	0
Expected.....	2.6	3.5	0.9
Mean winter production of ♀♀ in indicated class.....	37.50 eggs	14.00 eggs	

C. With 1 ♀ indicated to be of class 4 = $fl_1l_2 \cdot FL_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	2	2	0
Expected.....	2	2	0
Mean winter production of ♀♀ in indicated class.....	40.50 eggs	23.00 eggs	

All ♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	7	12	1
Expected.....	8	19	2
Mean winter production.....	47.43 eggs	16.25 eggs	0 eggs

While the families in this case are small, the evidence of segregation in about the expected proportions is clear.

B.P.R. ♂ 556. Indicated constitution = $fL_1L_2 \cdot fl_1l_2$.

This male was hatched in 1908 and used in the breeding pens in 1909. He proved a good breeder and got a fairly large number of adult daughters which were tested in respect to fecundity in the laying year 1909-10. As noted above he was the sire of class 4 ♂ 566. The breeding history of ♂ 556 follows.

Matings: A. With 4 ♀♀ indicated to be of class 1 = $fL_1L_2 \cdot FL_1l_2$.

♀ Progeny			
Winter Production:	Under 30	Over 30	Zero
Observed.....	8½	10½	4
Expected.....	8.6	11.5	2.9
Mean winter egg production of all daughters in indicated class.....	43.75 eggs	19.60 eggs	0 eggs

V. With 9 ♀♀ indicated to be of class 2 = $fL_1L_2 \cdot FL_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	10½	10½	0
Expected.....	10.5	10.5	0
Mean winter egg production of all daughters in indicated class.....	47.00 eggs	19.60 eggs	

All ♀ Progeny			
Winter Production:	Over 30	Under 30.	Zero
Observed.....	19	21	4
Expected.....	19.1	22	2.9
Mean winter production.....	46.00 eggs	19.60 eggs	0 eggs

The agreement here between observation and expectation is indeed remarkably close, and with a fairly large progeny.

Summary of results of all matings of class 4 males. Proceeding as before we may bring together here the results for each particular gametic combination taking all individuals together. While class 4 males were not used as often in these experiments as those of class 7, still the numbers involved are sufficiently large to give quite definite evidence regarding the segregation of fecundity factors.

TABLE 17

Showing the results of all matings of class 4 ♂♂ × class 1 ♀♀
 $fL_1L_2 \cdot fl_1l_2 \times fL_1L_2 \cdot FL_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE			WINTER EGG PRODUCTION OF DAUGHTERS			
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult progeny
4	17	Observed	21	30	8	59
		Expected	22.1	29.5	7.4	
Mean winter egg production of all ♀♀ in indicated class..			48.85 eggs	16.34 eggs	0 eggs	

TABLE 18

Showing the results of all matings of class 4 ♂♂ × class 2 ♀♀
 $fL_1L_2 \cdot fl_1l_2 \times fL_1l_2 \cdot FL_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult progeny
3	16	Observed	21	16.5	0	38
		Expected	19	19	0	

Mean winter production of all
 ♀♀ in indicated class 50.38 eggs 16.69 eggs

TABLE 19

Showing the results of all matings of class 4 ♂♂ × class 4 ♀♀
 $fL_1L_2 \cdot fl_1l_2 \times fL_1l_2 \cdot FL_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult progeny
2	4	Observed	5	5	0	10
		Expected	5	5	0	

Mean winter production of all
 ♀♀ in indicated class 43.40 eggs 13.60 eggs

TABLE 20

Showing the results of all matings of class 4 ♂♂ × all classes of ♀♀
General Summary

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult progeny
4	43	Observed	51½	62½	11	125
		Expected	51.45	62.5	11.05	

Mean winter production of all
 ♀♀ in indicated class 47.94 eggs 15.34 eggs 0 eggs

No closer agreement between observation and expectation than is here shown could be expected. The results of the matings discussed in this section confirm completely the general conclusions reached above from an examination of the matings of class 7 males.

Matings of Barred Plymouth Rock males of class 3.

Males of this class have a gametic constitution $fL_1L_2 \cdot fl_1L_2$. That is, they are homozygous with respect to the second, or excess, production factor, and heterozygous with respect to the first. Two males of this type were used in the experiments.

B.P.R. ♂ 65. Indicated constitution = $fL_1L_2 \cdot fl_1L_2$.

This male was purchased in 1908 from Mr. Wesley B. Barton, Dalton, Mass. Nothing is known of his breeding so far as concerns fecundity, but in all probability no particular effort towards breeding for high egg productiveness had ever been made in the stock from which he came. He was bred as a cockerel in the season of 1908 with the results set forth below.

Matings: A. With 1 ♀ indicated to be of class 1 = $fL_1L_2 \cdot Fl_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	7	1	1
Expected.....	6.75	2.25	0
Mean winter production of all ♀ ♀ in indicated class.....	53.00 eggs	19.00 eggs	0 eggs

B. With 1 ♀ indicated to be of class 6 = $fl_1L_2 \cdot Fl_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	2	3	0
Expected.....	2.5	2.5	0
Mean winter production of all ♀ ♀ in indicated class.....	37.00 eggs	18.67 eggs	

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	9	4	1
Expected.....	9.25	4.75	0
Mean winter production.....	49.44 eggs	18.75 eggs	0 eggs

In this case, while the number of successful matings was small, the families were relatively large. In the case of ♀ 366, set down here as probably of class 6, it should be said that this conclusion as to her gametic constitution is reached from a study of her daughters' and granddaughters' behavior. Her own winter egg record was 33, which on this view is regarded as a somatic fluctuation from the L_1 (Under 30) class.

B.P.R. ♂ 68. Indicated constitution = $fL_1L_2 \cdot fL_1L_2$.

As in the case of ♂ 65 nothing is known regarding the breeding of this bird, it having been purchased from Mr. Geo. W. Hillson, of Amenia, N. Y., early in 1908. It was bred as a cockerel the same season. The only matings to get adult daughters were those with class 1 females. The breeding history is as follows:

Matings: A. With 4 ♀ ♀ indicated to be of class 1 = $fL_1L_2 \cdot fL_1L_2$.

<i>All ♀ Progeny</i>			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
<i>Observed</i>	13	5	2
<i>Expected</i>	15	5	0
Mean winter production of all ♀ ♀ in indicated classes.....	59.00 eggs	25.20 eggs	0 eggs

The facts regarding the two zero birds here are of interest. According to theory no bird of this class should appear from any of these matings. One of these zero birds, E192, laid her first egg March 4, 1909, and proved thereafter, during the reproductive period (March 1 to June 1) to be a fairly good layer, with a total production for the period of 51 eggs.

Her laying during this and the subsequent summer period both in respect to its amount and its distribution, impresses one as like that of a bird carrying L_1 , rather than like that of a 'genetic' zero winter layer lacking this factor. I am of the opinion that this was the case. This bird, on such a view, would represent an extreme physiological variant in respect to the beginning of laying. While apparently bearing L_1 this factor did not come to expression until much later than under normal circumstances.

The other zero bird was pathological in respect to her reproductive organs. She never laid an egg and died July 16, 1909.

The autopsy record is as follows, plainly showing that the zero record of this bird cannot be taken as any indication whatever of her gametic constitution in respect to fecundity.

Autopsy of E 318, July 16, 1909. Body weight, 1730 grams. Hatched March 31, 1908. Oviduct small: parts of it contained masses of hardened secretion. Ovary with no large oöcytes. One small yolk resorbing. Body cavity filled with masses of hard yolk enclosed in peritoneal sacs. Some of these masses were small and attached to mesentery. Some were large. A large mass filled the dorsal part of body cavity on left side pushing over by a neck and connected by this with a similar mass on right side. This was partly hollow and in the cavity the surface was covered with a fruiting fungus resembling *Penicillium*. The peritoneum covering the masses of hard yolk formed adhesions with the viscera so that the intestine and oviduct were a bundle of adhesions clinging to these yolk masses.

It is only possible to summarize separately class 3 ♂ matings for class 1 females. This is done in table 21.

TABLE 21

Showing the results of all matings of class 3 ♂♂ × class 1 ♀♀

$fL_1L_2 : fl_1L_2 \times fL_1L_2 : Fl_1l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF DAUGHTERS				Total adult progeny
♂♂	♀♀	Class	Over 30	Under 30	Zero	
2	5	Observed	20	6	3	29
		Expected	21.75	7.25	0	

Mean winter production of all

♀♀ in indicated class 56.90 eggs 24.17 eggs 0 eggs

TABLE 22

Showing the results of all matings of class 3 ♂♂ with ♀♀ of all classes

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF DAUGHTERS				Total adult progeny
♂♂	♀♀	Class	Over 30	Under 30	Zero	
2	6	Observed	22	9	3	34
		Expected	24.25	9.75	0	

Mean winter production of all

♀♀ in indicated class 55.09 eggs 22.33 eggs 0 eggs

All matings of class 3 males are summarized in table 22.

The evidence for the segregation of high and low fecundity, as measured by winter egg production is quite as clear from the matings of class 3 ♂♂ as from those of class 7 or class 4 ♂♂ previously considered.

Matings of Barred Plymouth Rock males of class 2. Four males of this class were used in the experiments. None of them got a large number of adult daughters. It will be noted from table 9 that males of this class (gametic formula $fL_1L_2 \cdot fL_1l_2$) should produce daughters with winter records 'Over 30' and 'Under 30' in equal numbers regardless of the type of females with which the mating is made. The only basis for classifying the females in such matings is then the breeding behavior of their progeny, and particularly their daughters.

B.P.R. ♂ 32. Indicated constitution = $fL_1L_2 \cdot fL_1l_2$.

This male was hatched from Station stock in the spring of 1907 and bred in 1908. Nothing is known of his ancestry except that his mother was a '200-egg' hen. His breeding history follows:

Matings: A. With 2 ♀♀ indicated to be of class 1 = $fL_1L_2 \cdot FL_1l_2$.

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	4	3	0
Expected.....	3.5	3.5	0
Mean winter production of all ♀♀ in indicated class.....	52.50 eggs	44.33 eggs	

B.P.R. ♂ 57. Indicated constitution = $fL_1L_2 \cdot fL_1l_2$.

This male was purchased from Pine Top Poultry Farm, Hartwood, N. Y. in 1908 and bred as a cockerel that year.

Matings: A. With 2 ♀♀ indicated to be of class 2 = $fL_1L_2 \cdot FL_1l_2$.

♀ Progeny			
<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	2	1	0
Expected.....	1.5	1.5	0
Mean winter production of all ♀♀ in indicated class.....	38.00 eggs	24.00 eggs	

B. With 2 ♀♀ indicated to be of class 3 = $fL_1l_2 \cdot FL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	4	3	0
Expected.....	3.5	3.5	0
Mean winter production of all ♀♀ in indicated class.....	62.75 eggs	23.00 eggs	

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	6	4	0
Expected.....	5	5	0
Mean winter production of all ♀♀ in indicated class.....	54.50 eggs	23.25 eggs	

B.P.R. ♂ 17. Indicated constitution = $fL_1l_2 \cdot fL_1l_2$.

This cockerel was hatched in 1907 from a '200-egg' mother and was bred in the spring of 1908.

Matings: A. With 2 ♀♀ indicated to be of class 3 = $fL_1l_2 \cdot FL_1l_2$.

Winter Production:	Over 30	Under 30	Zero
Observed.....	3	3	0
Expected.....	3	3	0
Mean winter production of all ♀♀ in indicated class.....	47.00 eggs	12.33 eggs	

B.P.R. ♂ 70. This cockerel was purchased from Mr. M. L. Chapman, Farmington, Conn., and used in the breeding season of 1908. Nothing is known of his previous history.

Matings: A. With 3 ♀♀ indicated to be of class 2 = $fL_1L_2 \cdot FL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	4	5	0
Expected.....	4.5	4.5	0
Mean winter production of all ♀♀ in indicated class.....	68.25 eggs	19.00 eggs	

5. With 2 ♀♀ indicated to be of class 3 = $fL_1l_2 \cdot FL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	2	1	0
Expected.....	1.5	1.5	0
Mean winter production of all ♀♀ in indicated class.....	51.50 eggs	25.00 eggs	

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	6	6	0
Expected.....	6	6	0
Mean winter production.....	62.67 eggs	20.00 eggs	

Summary of results of all matings of class 2 males. The summarized results of the above matings are set forth in table 23.

TABLE 23

Showing the results of all matings of class 2 ♂♂ × class 2 ♀♀

$$fL_1L_2 \cdot fL_1l_2 \times fL_1L_2 \cdot FL_1l_2$$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE			WINTER EGG PRODUCTION OF DAUGHTERS			
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult progeny
2	5	Observed	6	6	0	12
		Expected	6	6	0	

Mean winter egg production of

all ♀♀ in indicated class... 58.17 eggs 19.83 eggs

TABLE 24

Showing the results of all matings of class 2 ♂♂ × class 3 ♀♀

$$fL_1L_2 \cdot fL_1l_2 \times fL_1l_2 \cdot FL_1l_2$$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE			WINTER EGG PRODUCTION OF DAUGHTERS			
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult progeny
3	6	Observed	9	7	0	16
		Expected	8	8	0	

Mean winter production of all

♀♀ in indicated class... 55.00 eggs 18.71 eggs

TABLE 25

Showing the results of all matings of class 2 ♂♂ × all classes of ♀♀

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF DAUGHTERS				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult progeny
	13	Observed	19	16	0	35
		Expected	17.5	17.5	0	

Mean winter production of all

♀♀ in indicated class 55.47 eggs 18.31 eggs

It is clear that the four class 2 males produced a progeny generation, which, though relatively small in absolute numbers, agrees very closely in respect to its gametic distribution with the expected results.

Matings of a Barred Plymouth Rock male of class 8. Males of class 8 are homozygous with respect to the absence of the first production factor, and heterozygous for the second, their gametic formula being $fl_1L_2 \cdot fl_1l_2$. But one bird of this type was used in the experiments.

B.P.R. ♂ 26. Indicated constitution = $fl_1L_2 \cdot fl_1l_2$.

This was one of the original stock in 1908. Nothing further is known of his breeding than that he was the son of a bird that had laid 200 or more eggs in her pullet year. His dam must have been of class 1, since a class 2 ♀ could not produce a class 8 ♂. Male 26 was bred as a cockerel in 1908 with the following results:

Matings: A. With 2 ♀♀ indicated to be of class 1 = $fL_1L_2 \cdot fl_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed	2½	9½	5
Expected	4.25	8.5	4.25
Mean winter production of all			
♀♀ in indicated class	35.00 eggs	9.89 eggs	0 eggs

B. With 5 ♀♀ indicated to be of class 4 = $fL_1l_2 \cdot FL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	6	6	0
Expected.....	6	6	0
Mean winter productions of all ♀♀ in indicated class.....	60.00 eggs	15.83 eggs	

All ♀ Progeny

Winter Production:	Under 30	Over 30	Zero
Observed.....	8½	15½	5
Expected.....	10.25	14.5	4.25
Mean winter production.....	60.50 eggs	12.26 eggs	0 eggs

One of the five females (♀ 397) of class 4 actually laid 31 eggs in her winter period and hence was literally an 'Over 30' bird. There can be no doubt, however, that this record is merely a fluctuation, and that ♀ 397 is really a class 4 bird of the constitution indicated. This is shown by her progeny.

Matings of a Barred Plymouth Rock male of class 1. Males of class 1 are extremely interesting both from the theoretical and the practical standpoint, since they are homozygous with respect to the presence of both fecundity factors. In consequence, *all* the daughters of any male of this class, regardless of the females with which he is mated, should be high producers. In the course of the experiments here under discussion only one male of this type has been used in the breeding pens, and owing to an unfortunate accident he was available for breeding only during a single season. This ♂ no. 550 was a remarkably fine and vigorous bird. He was easily the best bird, in respect to all fancy and utility points, out of the hundreds of cockerels raised the same year. He produced by the mating of a class 3 ♂ (♂ 68, p. 000, supra) and a class 1 ♀. That is,

$$\begin{array}{c}
 (\sigma 68) \ fL_1L_2 \cdot fL_1L_2 \times fL_1L_2 \cdot FL_1l_2 \ (\varphi 161) \\
 \downarrow \\
 \sigma 550 \\
 fL_1L_2 \cdot fL_1L_2
 \end{array}$$

This is a particularly interesting pedigree to anyone acquainted with the practical breeding and breeders of Barred Plymouth Rocks in this country, because, as already pointed out, ♂ 68 was purchased from Mr. G. W. Hillson, of Amenia, N. Y. Now it is generally supposed, and indeed has been stated by Mr. Hillson in his advertising, that his stock was founded from Mr. E. B. Thompson's 'Ringlet' strain, a stock very well known for quality in color and barring, but not commonly believed to be of any particular value for utility purposes. Yet here we have produced from this strain a male bird of the highest possible utility value, namely one that gets high-producing daughters regularly and without fail, regardless of the females to which he may be mated.

The breeding history of ♂ 550 is as follows:

- Matings:* A. With 5½ ♀♀ indicated to be of classes 1 or 2 = $fL_1L_2 \cdot FL_1l_2$ or $fL_1l_2 \cdot FL_1L_2$.
 B. With 4½ ♀♀ indicated to be of classes 3, 4, or 6 = $fL_1l_2 \cdot FL_1l_2$ or $fL_1L_2 \cdot FL_1l_2$ or $fL_1l_2 \cdot FL_1L_2$.

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	16½	½	1
Expected.....	18	0	0
Mean winter production of all ♀♀ in indicated class.....	51.25 eggs	¹	0 eggs

¹ See p. 184 for explanation of the convention of dividing the birds which lay exactly 30 eggs in the winter period.

The one daughter (F379) with the zero record was evidently abnormal in respect to her reproductive organs. During the last days of September and early October she began and kept up for a period of over a week daily visits to the nest (cf. section on "Matings of Barred Plymouth Rock males with Barred F_1 females") This is *normally* a *sure* indication of approaching laying. Further, birds which begin in this way not only are precocious in laying but make high winter records. This bird, however, stopped at once, and neither visited a nest, nor laid until late the next spring and then laid only a few eggs. There is little doubt that in this case the hereditary basis for high production was present (L_1L_2) but failed of expression for purely physiological reasons. Unfortunately no post-mortem examination of this

bird was made, the fact of her abnormality not having been recognized until too late to make such an examination possible.

It was unfortunate that ♂ 550 could not have been used during several breeding seasons. Even with the limited progeny actually available, however, the contrast between this bird and the others which have been discussed above is sufficiently striking.

Doubtful cases. In 1908 a Barred Plymouth Rock ♂ No. 61 was successfully mated with 3 ♀♀. The winter production records were as follows:

<i>Mothers' winter production</i>		<i>Daughters' winter production</i>		
♀ D157	Under 30	1 over 30	0 under 30	0 zero
♀ D168	Over 30	2 over 30	10 under 30	1 zero
♀ D90	Under 30	2 over 30	5 under 30	0 zero

The case is a difficult one, because of the behavior of certain of the daughters in subsequent matings. The most probable interpretation of the facts is that ♂ 61 belonged to class 4, and that D168 is a ♀ of class 1, but that in certain of her daughters bearing L_2 , this character did not come to full expression, giving a winter record of under 30. Three of the 10 daughters of ♀ D168 recorded as 'Under 30' laid 25 or more eggs in the winter period. If we suppose these to be really L_2 birds, we should then have the following *gametic* distribution of 168's daughters.

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	5	7	1
Expected.....	4.5	6.5	2

This is as close as could be expected.

Female D90 may be of either class 3 or 4. The data at hand do not enable one to determine with certainty between these possibilities. Female D157's only daughter left no adult ♀ offspring, and therefore it is not possible to make any conjecture as to her constitution, beyond the fact that she was probably not of class 1 or 2.

In the case of a number of males the families of adult daughters obtained were so small in size as to make impossible any accurate determination of the gametic constitution of the mothers used. All of these cases are here grouped together in one table.*

TABLE 26

Showing the results in respect to fecundity of daughters from pure Barred Rock matings in which the families were too small in size or number to permit classification as to gametic constitution

	NO. OF ♀ MATED	DAUGHTERS' WINTER PRODUCTION		
		Over 30	Under 30	Zero
(n)	5	6	4	1
16	2	4	1	0
5	2	3	1	0
11	14	14	11	1
527	5	4	4	0
555	8	5	8	0
551	11	9	8	1
Totals.....	47	45	37	3

It will be seen from this table that these families had on the average fewer than two adult daughters each, too small a number with which to work. This makes clear again the difficulty with which one has always to contend in practice in work with fecundity, namely that of getting even reasonably large families of normal *adult* daughters. One hatches a large number of chicks in order to supply thieves, crows, rats, hawks, etc., and finally get a small number of adult females available for the study of fecundity. Fecundity in fowls is not, as has been pointed out before, in all respects an ideal character for the investigation of the laws of inheritance.

Summary of results of all pure Barred Rock matings

The data presented in detail in this section of the paper, which deals with the matings of Barred Plymouth Rock males and females inter se, would appear to demonstrate the following points.

1. That there is a definite and clean-cut segregation (in the Mendelian sense) of high fecundity and low fecundity, the character 'fecundity' being here measured by winter egg production. The mode of inheritance is such as to indicate that winter egg

production depends upon two separately inherited physiological factors. The presence of both of these factors (L_1 and L_2) is essential to a *high* fecundity record. The second factor L_2 , without which high fecundity never appears is inherited in a sex-correlated manner, such that it is never borne in the same gamete that carries the female sex-factor F .

2. That the things segregated are perfectly definite and distinct. This is shown by the mean or average production records of the birds falling into the several fecundity classes. The birds bearing the factors for high fecundity have mean winter production records ranging from *two to five or six times as great* as the mean production records of birds lacking these high fecundity factors. Such differences as these do not depend upon refined statistical analysis for their detection and appreciation.

While by no means all the possible gametic combinations in respect to fecundity within the Barred Rock breed have yet been made, still the range covered by the data given above is fairly wide. All classes of females except the zero producers (class 5) have been repeatedly tested in the breeding pens in various different combinations. The zero winter producing females have been fairly often bred, but the difficulties of getting chickens hatched within the necessary time limits and in sufficient number to get adult daughters for fecundity work have been too great for the available resources. Of the nine possible types of males six have been tested in various combinations.

It may fairly be said, I think, that in its range, its quality and its amount, the evidence from the pure Barred Rock matings, as set forth in the preceding sections, is sufficient alone to demonstrate the Mendelian inheritance of fecundity in the breed of fowls. If, however, the principles set forth above for Plymouth Rocks are true, they ought to apply, in general at least, to other breeds of fowls and to crosses, with, of course, possible limitations and modifications in particular instances. It is desirable, therefore, to examine the results regarding the inheritance of fecundity in other breeds and crosses. This we may proceed to do.

Cornish Indian Game matings

The strain of Cornish Indian Game fowls used in these experiments is characterized, as has already been pointed out, by very poor egg production. There is no evidence that any of the individuals, either male or female, ever carry the second fecundity factor L_2 . These birds therefore represent the extreme condition in the way of low fecundity as compared with the Barred Plymouth Rocks.

The Cornish Indian Game is an old breed and if one may judge from poultrymen's accounts, there certainly have existed in the past, and probably exist now strains of birds belonging to this breed which are fairly good layers. Such strains, which are in marked contrast to the one here used, undoubtedly carry, in some combination, the second fecundity factor L_2 . There is nothing extraordinary, or contradictory to the results of the present paper, in such a fact (if it be a fact). Indeed it will be shown, in a later section of this paper, how it has been possible experimentally to form synthetically high laying Game hens, i.e., to put the factor L_2 into their hereditary constitution (cf. section on F_2 matings).

Owing to limitation of space and for other reasons it has not been possible to carry on the fecundity studies with this breed on anything like the same scale as with the Barred Rocks. Therefore the numbers here dealt with will be smaller than in the previous section. They will be sufficient, however, to indicate clearly the hereditary constitution of the material. Of the possible types of C.I.G. $\sigma\sigma$ in respect to fecundity as set forth in table 7, two (class 2 and class 3) have actually been used in pure Cornish matings (i.e., C.I.G. $\sigma \times$ C.I.G. φ).

Matings of a Cornish Indian Game male of class 2 (table 7). This σ , no. 558, was hatched in the spring of 1908 and used to head a pure Cornish pen in 1909. His breeding record indicated that he was of class 2, with a constitution $fL_2l_2 \cdot fl_1l_2$. His breeding history was as follows:

Matings: A. With 5 ♀♀ indicated to be of class 1 (Table 8) = $fl_1l_2 \cdot Fl_1l_2$.

♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	1	9	0
Expected.....	0	10	0
Mean winter production of all ♀♀ in indicated class.....	37.00 eggs	8.56 eggs	

B. With 2 ♀♀ indicated to be of classes 2 or 3 = $fl_1l_2 \cdot Fl_1l_2$ or $fl_1l_2 \cdot Fl_1l_2$.

♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	0	5	3
Expected.....	0	6	2
Mean winter production of all ♀♀ in indicated class.....		8.00 eggs	0 eggs

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	1	14	3
Expected.....	0	16	2
Mean winter production.....	37.00	8.36 eggs	0 eggs

The one bird recorded here as 'Over 30' laid but 37 eggs. Her progeny show clearly and unmistakably that she did not bear L_2 . That is to say, her record is a somatic fluctuation above the 30 limit, and has no gametic foundation. The agreement between observation and expectation on a gametic basis is really perfect for mating A. Taken as a whole the facts speak for themselves. The contrast with the results of Barred Rock matings is striking.

Matings of a Cornish Indian Game male of class 3 (table 7). Male no. 578 was hatched in 1909 and used in the breeding pens the following year. His breeding record shows that he was homozygous with respect to the absence of both fecundity factors, having the constitution $fl_1l_2 \cdot fl_1l_2$. He then belongs to class 3 of table 7. His breeding record is as follows:

Matings: A. With 4 ♀♀ indicated to be of classes 2 or 3 = fl_1l_2 . FL_1l_2 or fl_1l_2 FL_1l_2 .

♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	1	9	8
Expected.....	0	9	9
Mean winter production of all ♀♀ in indicated classes.....	39.00 eggs	13.11 eggs	0 eggs

B. With 1 ♀ indicated to be of class 4 = fl_1l_2 . FL_1l_2 .

♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	0	0	4
Expected.....	0	0	4
Mean winter egg production of all ♀♀ in indicated class.....			0 eggs

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	1	9	12
Expected.....	0	9	13
Mean winter production.....	39.00 eggs	13.11 eggs	0 eggs

Here again as in the previous case the single 'Over 30' record is a somatic fluctuation, with gametic significance. Leaving this out of account, or rather putting it in the 'Under 30' class where it belongs, the agreement between observation and expectation is very close.

Summary of results of Cornish Indian Game matings

Summarizing all pure Cornish matings which involved 2 ♂♂ and 20 ♀♀ (C.I.G. ♂ × C.I.G. ♀) we have the following results:

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	2	23	15
Expected.....	0	25	15
Mean winter production.....	38.00 eggs	10.22 eggs	0 eggs

Counting the two 'Over 30' records as somatic fluctuations belonging gametically to the 'Under 30' class the agreement between observation and theory is perfect. Thus it is seen that the same hypothesis which has been shown to account for the inheritance of fecundity in the Barred Plymouth Rock breed characterized in general by relatively high egg production, also accounts perfectly for the inheritance of this character in the entirely unrelated Cornish Indian Game breed, which is characterized by relatively poor egg production.

Reciprocal crosses of Barred Plymouth Rocks and Cornish Indian Games. F_1 generation

In connection with studies of the inheritance of plumage patterns and colors extensive experiments in crossing these two breeds have been carried out (cf. 40, 41). The results of these experiments in respect to fecundity form a crucial test of the validity of the Mendelian interpretation of the data from pure races set forth in the preceding pages. If the interpretation which has been given is correct it should account for the observed results in the F_1 , F_2 and subsequent cross-bred generations. Should it fail when subjected to this test, it would necessitate its acceptance with great reservation, if at all, for the pure races. On the other hand, agreement of the results from these cross-bred matings with those obtained from the pure-bred would afford the strongest confirmation which it is possible experimentally to obtain of the essential soundness of the general conclusions reached.

Matings of Barred Plymouth Rock males and Cornish Indian Game females. Two different males were used successfully¹³ in matings of this sort. Both of these birds were of class 7, having the gametic constitution fl_1L_2 . fl_1L_2 . One of them (≥ 554) was used in a number of pure B.P.R. matings with results already discussed in a previous section.

¹³ i.e., got adult daughters.

Mating of B. P. R. ♂ 559. Indicated constitution = $fl_1L_2 \cdot fl_1L_2$.

Matings: A. With 9 Cornish ♀♀ indicated to be of classes 2 or 3 = fl_1l_1 , FL_1l_2 or $fl_1l_2 \cdot FL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	18	17	2
Expected.....	18.5	18.5	0
Mean winter egg production of all ♀♀ of indicated class....	46.38 eggs	15.00 eggs	0 eggs

If we suppose the two zero birds to represent somatic fluctuations the agreement between observation and expectation is very close. Both these zero birds were late hatched and all the facts regarding them indicate that they carried L_1 , but for physiological reasons did not bring it to expression.

B. With 1 Cornish ♀ indicated to be of class 1 = $fl_1l_1 \cdot FL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	5	1	0
Expected.....	6	0	0
Mean winter egg production of all ♀♀ of indicated class....	42.75 eggs	20 eggs	

There is little doubt about this mating being of the type indicated, in spite of the one bird laying 'Under 30.' Her winter record was 20 eggs and she was a late (June) hatched bird. She probably carried L_2 , but this cannot be positively asserted because no male bird from her was mated. Only in this way could the point be settled.

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	23	18	2
Expected.....	24.5	18.5	0
Mean winter production.....	46.09 eggs	15.22 eggs	0 eggs

Matings of B. P. R. ♂ 554. This male has been shown above from his mating with Barred Rock females, to be of class 7 with the gametic constitution $fl_1L_2 \cdot fl_1L_2$. He was successfully mated

with one Cornish Indian Game ♀ of class 2. From this mating we have

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	1	3	0
Expected.....	2	2	0
Mean winter production.....	49 eggs	19.33 eggs	0 eggs

Putting all the results together we have for *all matings of B.P.R. ♂ × C.I.G. ♀ ♀*

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	24	21	2
Expected.....	26.5	29.5	0
Mean winter production.....	46.22 eggs	15.86 eggs	0 eggs

We see here the same agreement between observation and expectation which has appeared in the previous cases with pure matings.

Attention may next be turned to the reciprocal cross.

Matings of Cornish Indian Game males and Barred Plymouth Rock females. Four different Cornish males were used in these matings and got adult daughters. Their breeding histories follow.

Matings of C.I.G. ♂ 558. This bird was used in pure Cornish matings and there shown to be of class 2 (C.I.G.) with constitution $fL_1l_2 \cdot fl_1l_2$. His breeding history in producing F_1 females was as follows:

Matings: A. With 2 B. P. R. ♀ ♀ indicated to be of class 1 = $fL_1l_2 \cdot Fl_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	4	14	2
Expected.....	0	15	5
Mean winter production of all ♀ ♀ in indicated class.....	51.75 eggs	15.79 eggs	0 eggs

The four birds with 'Over 30' records are apparently outstanding exceptions. It should be noted that these birds came from mothers whose gametic constitution was of the general type $L_1 \cdot L_2$. This would seem to suggest that in this case the presence of L_2 in the mother, even though it did not pass to any of the F -bearing gametes, nevertheless in some manner or other modified the L_1 in these gametes so that a higher production in the progeny resulted. In other words, these cases suggest 'intra-zygotic influence' of the gametic factors upon one another, such as is frequently suggested by the conditions observed in heterozygotes, and lately has been discussed by Davenport (6, 7) and Laughlin (23). The winter records of these four birds are to be regarded as wide fluctuations, since when bred they gave no indications of carrying F_2 .

B. With 1 B.P.R. ♀ indicated to be of class 3 = $fL_1l_2 \cdot fL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	0	3	1
Expected.....	0	3	1

Mean winter production of ♀ ♀
in indicated class..... 6.00 eggs 0 eggs

All ♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	4	17	3
Expected.....	0	18	6
Mean winter production	51.75 eggs	14.06 eggs	0 eggs

Matings of C.I.G. ♂ 557. Indicated constitution: class 3
(C.I.G.) ♂ = $fl_1l_2 \cdot fl_1l_2$.

Matings: A. With 6 B.P.R. ♀ ♀ indicated to be of class 2 = $fL_1L_2 \cdot fL_1l_2$.

♀ Progeny

Winter Production:	Over 30	Under 30	Zero
Observed.....	0	17	0
Expected.....	0	17	0
Mean winter production of ♀ ♀ in indicated class.....		12.88 eggs	

15. With 1 B.P.R. ♀ indicated to be of class 4 = $fL_1l_2 \cdot FL_1l_2$.

♀ Progeny			
Winter Production:	Over 30.	Under 30	Zero
Observed.....	0	1	0
Expected.....	0	1	0
Observed winter production ..		10 eggs	

All ♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	0	18	0
Expected.....	0	18	0
Mean winter production.....		12.72 eggs	

The accordance between observations and expectation here is perfect.

Mating of C.I.G. ♂ 529. Indicated constitution: class 2 (C.I.G. ♂) = $fL_1l_2 \cdot fl_1l_2$.

Matings: A. With 1 ♀ indicated to be of class 1 = $fL_1L_2 \cdot FL_1l_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	1	1	1
Expected.....	0	2.25	0.75
Observed winter production. . .	46 eggs	13 eggs	0 eggs

This mating by itself is, of course, without any particular significance.

B. With 6 ♀ indicated to be of class 2 = $fL_1L_2 \cdot FL_1l_2$

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	5	12	0
Expected.....	0	17	0
Mean winter productions of all ♀ in indicated class	41.60 eggs	11.67 eggs	

Here, again, as in the case of ♂ 558 there are seen to be several birds with winter records of over 30 eggs, when none is expected.

C. With 3 ♀♀ indicated to be of class 7 = $fl_1L_2 \cdot FLl_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	1	3	2
Expected.....	0	3	3
Mean winter production of all ♀♀ in indicated class.....	45 eggs	13.00 eggs	0 eggs

All ♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	7	16	3
Expected.....	0	22.25	3.75
Mean winter production.....	42.71 eggs	12.00 eggs	0 eggs

The seven birds with records 'Over 30' belong gametically to the 'Under 30' class, and their records are somatic fluctuations. This is shown both by their history and by their behavior in F_2 , all having been bred.

Matings of C.I.G. ♂ 578. This male has been shown from his matings with pure Cornish females to belong to class 3 of C.I.G. ♂♂ (= $fl_1l_2 \cdot fl_1l_2$). His matings with Barred Rock females are as follows:

Matings: A. With 2 B.P.R. ♀♀ indicated to be of class 2 = $fl_1L_2 \cdot FLl_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	0	3	0
Expected.....	0	3	0
Mean winter production of daughters.....		6.33 eggs	

B. With 1 ♀ indicated to be of class 4 = $fl_1l_2 \cdot FLl_2$.

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	1	4	0
Expected.....	0	5	0
Mean winter production of daughters in indicated class..	42 eggs	11.00 eggs	0 eggs

All ♀ Progeny

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	1	7	0
Expected.....	0	8	0
Mean winter production.....	42 eggs	9.00 eggs	

There are no data from which to make sure whether the one bird with an 'Over 30' record represented fluctuation from the 'Under 30' class. It probably did, but this cannot be positively asserted.

Summary of all F_1 matings

Putting together the results of the matings of all Cornish Indian Game males with Barred Rock females, we have for the actual observations:

♀ Progeny—Raw Data

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	12	58	6
Expected.....	6	66.25	9.75
Mean winter production.....	45.67 eggs	12.46 eggs	0 eggs

In view of the fact that the 11 of the 12 birds with 'Over 30' records represent somatic fluctuations from the 'Under 30' class it is desirable to present another summary table in which the progeny are distributed in accordance with their gametic constitution.

♀ Progeny on Gametic Basis

<i>Winter Production:</i>	<i>Over 30 (L_1L_2)</i>	<i>Under 30 (L_1l_2)</i>	<i>Zero (l_1l_2)</i>
Observed.....	1	69	6
Expected.....	0	66.25	9.75

The contrast between these distributions and those of the reciprocal cross discussed before is very striking. Taken together these reciprocal crosses support strongly the general hypothesis of fecundity inheritance here being tested.

Matings of the second cross-bred (F_2) generation

The F_1 birds discussed in the preceding sections were mated in all possible ways *inter se* and with the parent forms. The results of these matings will be discussed in the present section.

At the outstart it should be noted that in spite of the fact that as many F_2 birds were hatched and reared as the available facilities would permit, nevertheless, the number of adult daughters available for fecundity study is small in case of some of the matings. There are several reasons for this. Besides the obvious one such as mortality, depredations of thieves, hawks, crows, rats and the like, there is another important but not so obvious one. This is the failure or great difficulty experienced in getting certain of the F_2 cross breeds to grow into normal, full-sized, healthy adult birds. After rather wide experience in handling cross-bred chicks, I am convinced that certain gametic combinations which are to be expected on Mendelian theory, and can be produced in the expected numbers in the breeding pen, are nevertheless physiologically abnormal or unsound. Such birds do not make a normal growth, but in spite of the best care and attention grow up into stunted weaklings, which always show, both in their structure and their physiological economy, the effect of this retarded, abnormal development. I am further convinced that this result is primarily due to the hereditary constitution of the individuals in question. Certain combinations of hereditary factors do not produce physiological sound and vigorous zygotes.

Of course, there is nothing novel in such a result. It is of a piece, for example, with the parts respecting the relation between hereditary constitution and physiological vigor in maize, which have been so clearly set forth and analyzed by Shull (45, 46, 47) and East (9). Other examples of the same phenomenon might be cited. The whole phenomenon is precisely what would be expected from Johanssen's general conception of inheritance and outogeny (22).

This relationship between hereditary constitution and physiological constitution or normality takes on particular significance when one is dealing with fecundity. As has been pointed out

earlier in this paper one cannot expect to get a normal somatic expression of the hereditary constitution in respect to fecundity unless the bird is a physiologically normal, well-developed individual. Stunted, under-developed, or physiologically unsound birds will lay but very few if any eggs, regardless of what fecundity factors it may carry. A marked difference is here apparent between structural and physiological characters so far as the study of inheritance is concerned. A definite structure either is or is not present in the zygote, however weak physiologically the individual may be. But if the general capability of an organism with respect to the transformation of matter and energy is markedly reduced, then all physiological characters will be affected, and fail to reach complete normal expression.

In the study of cross-bred poultry I have found pure extracted whites from crosses involving originally two heavily pigmented parent races to be conspicuously good examples of the phenomenon under discussion. It is only very exceptionally, in my experience, that such white birds are physiologically normal. Indeed because of this fact it is only with the greatest difficulty, and after many failures, that I have been able to get such extracted whites to breed, and thus form a pure white race. If the hens lay eggs, which some do not do, they are usually either infertile, or else all the embryos die at an early stage. These facts have some bearing on the popular belief of animal breeders that whites in general are delicate in constitution and hard to rear. This belief is so well known that it is not necessary to cite in detail references regarding it in the literature.

As a consequence of the above considerations, I have felt justified in leaving out of account, or rather in considering apart from the others, a few of the F_2 individuals, in all some 7 out of over 200 F_2 birds all told. In each case these birds were physiologically abnormal, and obviously so to the most casual observer. The fact that they did not lay was no criterion whatsoever of their hereditary constitution. In order that there might be no possibility of unfairly influencing ratios by leaving these birds out, the whole families (usually of two or three individuals only) to which they belonged have been rejected. As a matter of fact

whenever one individual in a family is physiologically abnormal in this way all the other members will usually show the same condition in greater or less degrees.

In the F_2 generation following the reciprocal crossing of Barred Rocks and Cornish Indian Games there are a number of possible matings. The nature of these matings and the results as to color and pattern have been discussed in another place (41). That paper may be referred to in case one is not clear as to the nature of the matings. The different matings will be discussed in the following order.

1. $F_1 \sigma$ (out of B.P.R. $\sigma \times$ C.I.G. φ) $\times F_1 \varphi \varphi$, barred and non-barred.
2. $F_1 \sigma$ (out of C.I.G. $\sigma \times$ B.P.R. φ) $\times F_1 \varphi \varphi$, barred and non-barred.
3. $F_1 \sigma$ (out of B.P.R. $\sigma \times$ C.I.G. φ) \times B.P.R. $\varphi \varphi$
4. $F_1 \sigma$ (out of B.P.R. $\sigma \times$ C.I.G. φ) \times C.I.G. $\varphi \varphi$
5. $F_1 \sigma$ (out of C.I.G. $\sigma \times$ B.P.R. φ) \times B.P.R. $\varphi \varphi$
6. $F_1 \sigma$ (out of C.I.G. $\sigma \times$ B.P.R. φ) \times C.I.G. $\varphi \varphi$
7. B.P.R. $\sigma \sigma \times F_1$ Barred $\varphi \varphi$
8. B.P.R. $\sigma \sigma \times F_1$ non-barred $\varphi \varphi$
9. C.I.G. $\sigma \times F_1 \varphi \varphi$ Barred and non-barred

It will be recalled that the *barred* F_1 females come from the mating B.P.R. $\sigma \times$ C.I.G. φ_1 and that the *non-barred* (*black*) F_1 females comes from the reciprocal mating C.I.G. $\sigma \times$ B.P.R. φ .

Matings of $F_1 \sigma$ 576 with F_1 females. The pedigree of $F_1 \sigma$ 576 was as follows:

$$\begin{array}{c} \text{B.P.R. } \sigma \text{ 559 } (fl_1L_2 . fl_1L_2) \times \text{C.I.G. } \varphi \text{ 456 } (fL_1l_2 . fL_1l_2) \\ \downarrow \\ F_1 \sigma \text{ 576} \end{array}$$

The hereditary constitutions of both σ 559 and φ 456 were known, both from their pedigrees and their progeny in other matings. From this pedigree it is evident that the gametic formula for σ 576 must be either $fl_1L_2 . fL_1l_2$ or $fL_1L_2 . fl_1l_2$. A study of his progeny in all matings shows clearly that it is actually the former. In other words he produced gametes of four kinds, viz., fl_1L_2 , fL_1L_2 , fL_1l_2 , fl_1l_2 .

This bird was mated with barred F_1 ♀♀ which had been produced by the following mating.

B.P.R. ♂ 559 ($fl_1L_2 \cdot fl_1L_2$) × C.I.G. ♀♀ of type $fl_1l_2 \cdot Fl_1l_2$

↓

A. $fl_1L_2 \cdot FL_1l_2$ —Winter record over 30

and

B. $fl_1l_2 \cdot Fl_1l_2$ —Winter record under 30

Male 576 was mated only with ♀♀ of the A class,¹⁴ namely those having a gametic constitution $fl_1L_2 \cdot FL_1l_2$, and producing two sorts of F -bearing gametes, Fl_1l_2 and FL_1l_2 .

This same ♂ 576 was mated with non-barred (black) F_1 females which had been produced in the following way.

C.I.G. ♂ 558) ×	B.P.R. ♀♀ some of which
or		were of type $fl_1L_2 \cdot Fl_1l_2$,
529 ($FL_1l_2 \cdot fl_1l_2$)		and some $fl_1l_2 \cdot FL_1l_2$ (both
) ↓	producing F -bearing gametes of two kinds FL_1l_2
		and Fl_1l_2)

A. $fl_1l_2 \cdot FL_1l_2$, Winter record under 30

B. $fl_1l_2 \cdot FL_1l_2$, Winter record under 30

C. $fl_1l_2 \cdot Fl_1l_2$, Winter record under 30

D. $fl_1l_2 \cdot Fl_1l_2$, Winter record zero.

The three non-barred ♀♀ with which ♂ 576 was bred were of the B-C type, producing two kinds of F -bearing gametes, FL_1l_2 and Fl_1l_2 . They were thus identical, so far as concerns F -bearing gametes with the barred F_1 birds with which ♂ 576 was mated. All the progeny may then be treated together since all did, as a matter of fact, lead to the same result, having regard to the errors of sampling in such statistically small lots.

¹⁴ It would of course be desirable to have data from the other mating, ♂ 576 × B ♀♀. If one could have foreseen what the mechanism of the inheritance of fecundity was going to turn out to be, such matings would have been made. Actually these cross bred birds were being studied primarily with reference to color characters, and the matings were made relative to that line of investigation. Naturally highly fecund females would be chosen as breeders whenever possible, in order to get more chickens for the color studies. Actually, however, all of the possible gametic combinations in respect to fecundity were tested in F_2 either from one mating or another.

TABLE 27

Showing the results of mating $F_1 \sigma$ 576 with $F_1 \varphi \varphi$
 Type of mating: $fl_1L_2 \cdot fl_1l_2 \times fl_1L_2 \cdot FL_1l_2$ and (or) $fl_1l_2 \cdot FL_1l_2$

INDIVIDUALS USED IN THESE MATINGS		WINTER RECORD OF DAUGHTERS			
σ	φ	Over 30	Under 30	Zero	Total and φ progeny
576	F68	4	2	0	6
576	F89	0	3	0	3
576	F41	1	3	0	4
576	F79	1	3	1	5
576	F416	0	0	2 ¹	2
576	F421	0	2	2	4
576	F33	5	3	2	10
576	F415	0	1	1	5
Total observed.....		11	20	8	39
Total Expected.....		14.6	19.5	4.9	
Mean winter production of $\varphi \varphi$ in indicated class....		42.81 eggs	12.05 eggs	0 eggs	

¹ These two individuals ought really to be excluded on the ground of physiological abnormality of the sort discussed at the beginning of this section. Neither of them made a normal growth. No poultrymen would have regarded these birds as reliable material for the study of egg production. Leaving these two birds out the totals stand as follows:

Winter Production:	Over 30	Under 30	Zero
Observed.....	11	20	6
Expected.....	13.9	18.5	4.6

The same kind of evidence for the segregation of different degrees of fecundity which has been seen in all the previous matings appears again in these F_2 birds.

Matings of $F_1 \sigma$ 577 with F_1 females. This $F_1 \sigma$ 577 was produced in the following way:

C.I.G. σ 558($fl_1l_2 \cdot fl_1l_2$) \times B.P.R. φ 234($fl_1L_2 \cdot FL_1l_2$)

↓

$F_1 \sigma$ 577

Such a mating as this would be expected to produce males of four (really three *different*) kinds as follows:

- | | |
|----------------------------|----------------------------|
| A. $fl_1l_2 \cdot fl_1L_2$ | C. $fl_1l_2 \cdot fl_1L_2$ |
| B. $fl_1l_2 \cdot fl_1L_2$ | D. $fl_1l_2 \cdot fl_1L_2$ |

The results indicate that ♂ 577 was of the last (D) type, producing two kinds of gametes, fl_1l_2 and fl_1L_2 . He was mated with 4 barred F_1 ♀ and 4 black F_1 ♀. All of these females, as in the preceding case, produced F -bearing gametes of two kinds in unequal numbers; Fl_1l_2 and FL_1l_2 . Of these matings three produced small families in which all of the individuals were so far from being normal physiologically that they cannot fairly be included in the tabulation. The details regarding them are as follows. From one barred F_1 ♀ was produced two adult daughters, both of which were undersized and stunted in development, and failed to lay. One of these daughters died early in the year. From one of the matings with black F_1 ♀ only one adult daughter was obtained, which again failed to develop normally and was only put into the adult house because of its interest from the standpoint of color inheritance. Another of the matings with a black F_1 ♀ produced four adult daughters. Two of these were extracted whites and very small, poor specimens. The whole family was saved because of these birds. Neither of them laid. Of the other sisters one died early in the laying year, never having laid. It, like the other members of the family, was from the start a weakling. Finally the fourth sister made a winter record of 8 eggs. It presented the same evidence of abnormality as the other sisters, and its egg record could by no means be taken as a just indication of its gametic constitution in respect to fecundity. No one of the seven birds under discussion would ever by any chance whatever have been put in the laying house as normal individuals for the study of fecundity. The only reason they ever were put in was simply, as already explained, because the primary object of the F_2 birds as a whole was the study of color and pattern inheritance. Even though a bird is an undeveloped weakling physiologically one may make a record of its plumage color and pattern, and see whether these change with advancing age. However, since these birds really were in the adult house, and in order to forestall the possibility of a suggestion that any records were suppressed in this study of fecundity, it has seemed advisable to take the space for the above detailed discussion of the matter.

The records for the other matings of $F_1 \sigma$ 577 are given in table 28.

Matings of $F_1 \sigma$ 576 with Barred Plymouth Rock females. This $F_1 \sigma$ was mated with three pure Barred Rock $\varphi \varphi$ of class 2 (table 6). The results of these matings are shown in table 29.

TABLE 28

Showing the results of mating $F_1 \sigma$ 577 with $F_1 \varphi \varphi$

Type of mating: $fl_1l_2 \cdot fl_1L_2 \times fl_1L_2 \cdot FL_1l_2$ and $fl_1l_2 \cdot FL_1l_2$

Gametes: fl_1l_2 and fl_1L_2 . F-bearing gametes: FL_1l_2 and FL_1l_2

INDIVIDUALS USED IN THESE MATINGS		WINTER RECORD OF DAUGHTERS			
σ	φ	Over 30	Under 30	Zero	Total adult φ progeny
577	F401	0	3	0	3
577	F18	0	2	1	3
577	F44	2	4	1	7
577	F99	2	2	0	4
577	F418	1	5	5	11
Total observed.....		5	16	7	28
Total expected.....		7	14	7	
Mean winter production of $\varphi \varphi$ in indicated class....		36.33 eggs	11.25 eggs	0 eggs	

TABLE 29

Showing the results of mating $F_1 \sigma$ 576 with Barred Rock $\varphi \varphi$

Type of mating: $fl_1L_2 \cdot fl_1l_2 \times fl_1L_2 \cdot FL_1l_2$

INDIVIDUALS USED IN THESE MATINGS		WINTER RECORD OF DAUGHTERS			
σ	φ	Over 30	Under 30	Zero	Total adult φ progeny
576	1107	1	1	0	2
576	F115	1	2	0	3
576	F111	7 $\frac{1}{2}$	3 $\frac{1}{2}$	0	11
Total observed.....		9 $\frac{1}{2}$	6 $\frac{1}{2}$	0	16
Total expected.....		8	8	0	
Mean winter production of $\varphi \varphi$ in indicated class....		46.78 eggs	22.83 eggs		

These results are suggestive in connection with the problem of the absolute fecundity value of the same genes from different sources, a matter which will be fully discussed later. The figures give clear evidence of Mendelian segregation of high and low fecundity.

Matings of F₁ ♂ 576 with Cornish Indian Game females. There were but two matings of ♂ 576 with pure Cornish Indian Game ♀ ♀ which produced adult female offsprings. Other matings were made but got no adult progeny. The successful matings were with C.I.G. ♀ ♀ of constitution $fl_1l_2 \cdot FL_1l_2$. The results were as follows:

σ 576 $fl_1L_2 \cdot fl_1l_2 \times fl_1l_2 \cdot FL_1l_2$			
♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	0	3	1
	0	2	0
	<hr/>		
Total observed.....	0	5	1
Total expected.....	1.5	3	1.5
Mean winter production of ♀ ♀ in indicated class.....		11.80 eggs	0 eggs

The numbers here are too small to give definite results, but there is nothing incompatible in the observations, having regard to the smallness of the numbers, with what would be expected.

Matings of F₁ ♂ 577 with Barred Plymouth Rock females. There were three matings of this sort, but the families were all small. The females used were of class 2 (table 6). The results were as follows:

σ 577 $fl_1l_2 \cdot fl_1l_2 \times fl_1L_2 \cdot FL_1l_2$			
♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
(♀ 1118).....	0	1	0
(♀ F350).....	1½	1½	0
(♀ F235).....	1	2	0
	<hr/>		
Total observed.....	2½	4½	0
Total expected.....	3.5	3.5	0
Mean winter production of ♀ ♀ in indicated class.....	35.00 eggs	11.25 eggs	

Again the numbers involved are too small to be of any particular significance when taken by themselves. They are in conformity, however, with all the other data and therefore have cumulative value.

Matings of $F_1 \text{ } \sigma 577$ with Cornish Indian Game females. Two matings only of this sort got adult female progeny. The families are small. The pure Cornish females used were of constitution $fl_1l_2 \cdot Fl_1l_2$. The results follow.

$$\sigma 577 \text{ } fl_1l_2 \cdot fl_1L_2 \times fl_1l_2 \cdot Fl_1l_2.$$

σ Progeny

Winter Production:	Over 30 .	Under 30	Zero
(\varnothing F7)	0	1	1
(\varnothing F11).....	0	2	1
Total observed.....	0	3	2
Total expected.....	1.25	2.5	1.25
Mean winter production of $\varnothing \varnothing$ in indicated class.....		7.33 eggs	0 eggs

Matings of Barred Plymouth Rock males with Barred F_1 females. While several matings were made here they all fell into one or the other of two gametic types. The Barred Rock males used in these matings have, of course, already had their gametic constitutions determined through their matings with pure Barred Rock females.

A. Type of mating: $fl_1L_2 \cdot fl_1L_2 \times fl_1L_2 \cdot FL_1l_2$. The results of this type of mating are shown in table 30.

There are several points which need to be noted about this table. While in general it is apparent that the observed result falls out in fair accord with expectation, the three zero birds are outstanding exceptions. No zero birds should occur in any of these matings. A careful study of the individual cases, however, indicates that only one of these apparent exceptions is really such. Two of these zero birds were very late hatched and began laying immediately after March 1, i.e., just after the arbitrary point of ending the winter period. Their records during the spring period were such as to indicate that they bore the factor L_1 , and not that

TABLE 30

Showing the results of mating Class 2 B.P.R. ♂♂ with high laying Barred F₁ ♀♀

MATING		OBSERVED WINTER PRODUCTION OF DAUGHTERS		
B. P. R. ♂	Barred F ₁ ♀	Over 30	Under 30	Zero
552	424	3	5	0
554	87	0	4	0
563	412	7	4	0
564	405	7	2	0
567	425	2	1	3
562	404	$\frac{1}{2}$	$\frac{1}{2}$	0
Total observed.....		19 $\frac{1}{2}$	13 $\frac{1}{2}$	3
Total expected.....		18	18	0
Mean winter production.....		57.16 eggs	12.69 eggs	

they lacked it. The third zero bird (no. 558) was an extremely interesting case. Something was at fault physiologically with her reproductive organs, as a result of which she never laid an egg. However, she gave clear evidence that she bore the factor L_1 (or L_2 , not both) gametically. This she did through her nesting records. For some years past records have been kept in this work not only of when a hen visited a nest and laid, but also when she visited a nest and did not lay. A large number of records have been accumulated of birds which go through the whole process of nesting and laying except that they do not discharge any eggs from the body. It was hoped to publish a paper on this subject, which throws light on the problem of the physiology of egg production, before the present paper appeared. This has not been possible owing to pressure of other work, so it will be necessary to take a little space here to discuss certain phases of this subject. It has been shown experimentally in the laboratory that if the oviduct of a normal healthy hen, with a normal ovary, is ligated, transected or removed entirely, without injury to the ovary, such a bird goes regularly through the entire process of laying, save for the extrusion of an egg, which is physically impossible. The 'n' (nesting) record of such a bird is precisely like a normal egg record, showing the same phenomena of rhythm and

cycles. Each day's 'n' in the record of such a bird represents an egg¹⁵ which she would have laid, had she been physically capable of so doing.

Birds in which the oviduct is occluded through some diseased condition often behave in this same manner. It may result from other abnormal conditions also. With this explanation, the following record of ♀ 558, will be clear, it being understood that 'n' denotes a visit to a nest and the performance of those acts characteristically associated with the laying of an egg, but without the extrusion of an actual egg.

Date.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Nov.																															
Dec.																															
Jan.											n								n	n	n	n	n	n	n	n	n	n	n	n	n
Feb.			n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Mar.	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Apr.	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
May	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Jun.	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Jul.	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Aug.	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n

Fig. 3 Nesting record of ♀ 558. 'n' denotes a visit to the trap-nest. 'n' indicates that the bird visited the nest twice on the same day. B indicates that the bird became broody, and O that she ceased manifestations of broodiness.

From this record taken in connection with other similar cases studied in this laboratory, there can be no doubt that ♀ 558 belongs gametically in the class 'Under 30.'

Another point is with reference to the mating ♂ 564 × ♀ 405. The excess of 'Over 30' birds here is in part due to the fact that two birds, which have winter records respectively of 32 and 34 eggs and are almost certainly to be regarded as somatic fluctuations above the division point at 30 are included in the 'Over 30' class.

¹⁵ Of course this does not mean that when a bird visits a nest twice in the same day she would have laid two eggs that day had she been normal. Many laying birds have the habit of visiting the nest once or twice in the same day before actually laying.

If the totals are modified in accordance with the above suggestion we get

<i>Winter Production</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed: (modified).....	17½	17½	1
Expected.....	18	18	0
Mean winter production:.....	60.00 eggs	15.40 eggs	0 eggs

$$\left. \begin{array}{l} \text{B. Type of mating: } fL_1L_2 \cdot fl_1l_2 \\ \text{or} \\ fL_1l_2 \cdot fl_1L_2 \end{array} \right\} \times fl_1L_2 \cdot FL_1l_2$$

♀ Progeny

MATING		OBSERVED WINTER PRODUCTION OF DAUGHTERS		
B. P. R. ♂	Barred F ₁ ♀	Over 30	Under 30	Zero
506	419	2	3	1
Expected.....		2.25	3	0.75
Mean winter production.....		46.50 eggs	13.67 eggs	0 eggs

While this single family is small the three classes expected are represented and in as near the right proportion as could be expected.

Putting together all the results for B.P.R. ♂♂ × Barred F₁ ♀♀ we get:

Unmodified Data

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	21½	16½	4
Expected.....	20.25	21	0.75

Data modified in accordance with physiological facts regarding individual birds

<i>Winter Production:</i>	<i>Over 30</i>	<i>Under 30</i>	<i>Zero</i>
Observed.....	19½	20½	2
Expected.....	20.25	21	0.75

Matings of Barred Plymouth Rock males and F₁ Black females.
A number of matings of this type were made, representing several

different gametic combinations. The following B.P.R. males were used in these matings. The class and gametic constitution given are those which have been brought out by the pure B.P.P. matings.

BIRD NO.	CLASS (TABLE 5)	GAMETIC CONSTITUTION
552	7	$f l_1 l_2 \cdot f l_1 l_2$
554	7	
562	7	
573	7	
564	7	
567	7	$f L_1 l_2 \cdot f l_1 l_2$
569	4	

Of these males four, namely 552, 554, 573 and 564, were mated with females whose sire was C.I.G. ♂ 557. These birds all had the gametic constitution $f l_1 l_2 \cdot F L_1 l_2$, as shown in the section on the F_1 birds. The results of these matings are shown in table 31.

The observed figures are a rather bad, though not an impossible, approximation to the expected Mendelian half. The means indicate, however, that there is a definite segregation of the two classes. It is possible that the discrepancy in the ratio finds its explanation in a difference in the potency or absolute fecundity value of the Cornish Indian Game L_1 factor and the same factor in the Barred Plymouth Rock.

TABLE 31
Showing the results of matings of the type B.P.P. ♂ $f l_1 l_2 \cdot f l_1 l_2$
× Black F₁ ♀ $f l_1 l_2 \cdot F L_1 l_2$

NUMBER OF INDIVIDUALS INVOLVED IN MATINGS OF THIS TYPE		WINTER EGG PRODUCTION OF DAUGHTERS F_2				
♂♂	♀♀	Class	Over 30	Under 30	Zero	Total adult ♀ progeny
4	4	Observed	5	14	0	19
		<i>Expected</i>	<i>9.5</i>	<i>9.5</i>	<i>0</i>	
Mean winter egg production of all ♀♀ in indicated class.....			37.00 eggs	11.14 eggs		

Two of the class 7 B.P.R. males (562 and 567) were mated with two F_1 blacks whose sire was C.I.G. ♂ 558. It has already been shown from the pure Cornish and F_1 matings that this Cornish ♂ 558 had the gametic constitution $fL_1l_2 : fl_1l_2$. In respect to fecundity his F_1 daughters in the 'Under 30' class were gametically of two types: viz., $fL_1l_2 : Fl_1l_2$ and $fL_1l_2 : FL_1l_2$. None of the second type were used in these matings. Only a small progeny resulted from the mating of the two females of the first type. The actual results were as follows:

♀ Progeny			
Winter Production:	Over 30	Under 30	Zero
Observed.....	2	4	0
Expected.....	3	3	0
Mean winter egg production of			
all ♀♀ in indicated class....	38.00 eggs	8.5 eggs	

B.P.R. ♂ 569 was mated with a black F_1 ♀ sired by C.I.G. ♂ 529. Only two daughters were obtained. Both made winter records under 30 eggs. The number of daughters is too small to have any significance, or to make classification possible.

Putting all the results together (with the exception of the two individuals just noted as not capable of classification) we have:

F_1 ♀ Progeny from matings of B.P.R. ♂♂ × Black F_1 ♀♀			
Winter Production:	Over 30	Under 30	Zero
Observed.....	7	18	0
Expected.....	12.5	12.5	0
Mean winter production.....	37.29 eggs	10.55 eggs	

There is clear evidence of segregation here but there is a defect in the observed numbers in the 'Over 30' class. After careful study of all the facts a possible explanation of this appears to me that the absolute degree of fecundity manifested somatically when L_1 is present in the gametes may be less if the L_1 comes from a Cornish Indian Game than if it comes from a Barred Plymouth Rock. In other words it appears to be the case that what may be called the absolute fecundity value or worth of L_1 is different in these two breeds. An indication that this is the case is found in

the following figures. In the case of pure C.I.G. matings the factor L_2 is not present. All females in the 'Under 30' class are therefore either L_1l_1 or L_1L_1 in type. The mean winter production of all pure C.I.G. ♀♀ in the 'Under 30' class is 10.22 eggs. The mean winter production of all pure B.P.R. ♀♀ in the 'Under 30' class is 15.61 eggs. The probable errors in both cases are less than 1. Of course the 'Under 30' class in the case of B.P.R. ♀♀ includes the following gametic types: L_1l_1 , L_1L_1 , l_1l_1 , and L_2l_2 . The last two do not occur in the pure C.I.G.'s. Granting the greatest conceivable influence to this, it is still evident that the L_1 factor of a Barred Rock probably means a higher winter production than the L_1 of a Cornish Game. But if this is true then plainly the division or upper limiting point for the low fecundity class should not be at 30 eggs but at some lower point in the case of females bearing L_1 from a Cornish Indian Game source. If it be kept at 30 eggs for all birds, and there is a difference in the absolute fecundity value of the factors, then plainly some birds will be put in the low fecundity class, because of an 'Under 30' record, although they really carry L_2 and belong in the high fecundity class. Such a state of affairs would account for just such discrepancies as those observed in the matings under discussion.

Matings of Cornish Indian Game males with Black and Barred F_1 females. Only one C.I.G. male was used in these F_2 back-cross matings. This was C.I.G. ♂ 578. As will presently appear, it is to be regretted that other Game males were not used, because of certain peculiarities arising in the results of some of these matings. From pure Cornish and F_1 matings ♂ 578 has been shown to have the gametic constitution fl_1l_2 . fl_1l_2 .

Male 578 was mated with 3 black F_1 females indicated to be of the type fL_1l_2 . FL_1l_2 , with the following results:

Matings: C.I.G. ♂ fl_1l_2 . fl_1l_2 × F_1 ♀ fL_1l_2 . FL_1l_2 .

	♀ Progeny		
Winter Production:	Over 30	Under 30	Zero
Observed	1	9	1
Expected	0	11	0
Mean winter production	41 eggs	9.67 eggs	0 eggs

It will be noted that the one 'Over 30' bird laid but 41 eggs. The record probably represents a fluctuation from the 'Under 30' class. In general the agreement between observation and expectation is satisfactory.

Turning to the matings of ♂ 578 with barred F_1 females we meet the only case in the whole investigation which is apparently uncomfortable. It will therefore be well to discuss it in detail. The facts are these: ♂ 578 was mated with four barred F_1 females, of which three were in the 'Over 30' class and one was a poor layer. When mated with any of these the high laying birds one half of 578's daughters should have been in the 'Over 30' and one-half in the 'Under 30' class. Mated with the poor layer only zero birds should have resulted. Nothing like this actually happened. The observed outcome was that shown in the following table:

Mating 699 (♂ 578 × ♀ 411) gave 13 adult daughters, with winter records as follows: 62, 60, 56, 32, | 30, | 28, 27, 26, 26, 7, 5, 5, 2.

Mating 700 (♂ 578 × ♀ 422) gave 7 adult daughters with winter records as follows: 47, 47, 33, | 26, 19, 15, 9.

Mating 701 (♂ 578 × ♀ 414) gave 5 adult daughters with winter records as follows: | 23, 16, 5, 0, 0.

Mating 702 (♂ 578 × ♀ 423) gave 11 adult daughters with winter records as follows: 40, 34, | 28, 26, 21, 20, 17, 15, 13, 10, 4.

These records are characterized by four striking facts: (a) the large number of 'Over 30' records when none is expected, (b) the large number of high 'Under 30' records, (c) the absence except in one mating of zero records, and (d) the sharp break within the 'Under 30' class, especially to be noted in mating 699, but also clearly in each of the others.

Now these four matings were remarkable in other respects than the egg records of the progeny. They gave an extraordinarily high hatching record. This is shown in table 32.

Considering that these figures include all eggs set during the whole hatching season it is evident that the record is relatively very high. In a former paper (30) I have shown (*loc. cit.*, table B, p. 131) that for the high laying Barred Rock matings the mean percentage of fertile eggs was 80.7 per cent., while 55.1 per cent of the fertile eggs were hatched. Even those results could only be

TABLE 32

Showing the hatching records of ♂ 578's F₂ matings

MATING	EGGS SET	EGGS INFERTILE	EGGS FERTILE	CHICKS HATCHED
699	58	0	58	46
700	57	0	57	37
701	37	1	36	27
702	49	5	44	37
Totals....	201	6	195	147

Per cent of eggs fertile = 97.01.

Per cent of fertile eggs hatched = 75.38.

considered very good taking all the facts into consideration. These back-cross matings of ♂ 578 far surpass those records.

One can only conclude that for some reason not apparent the matings 699 to 702 were physiologically extremely favorable. There seems to have been a what the breeders call a 'nick' here of unusual character. These matings were noticeable throughout the hatching season not only for the large number of the chicks produced, but also for their extra fine, vigorous character. The chick mortality from these matings was low.

As has been pointed out at the beginning of this section of the paper, there undoubtedly exist differences in what might be called physiological compatibility between fowls of different genotypic constitutions. Some concrete data regarding this have been published in a previous paper (39). More will be presented later. While some gametic combinations (at least at their first synthesis) lead to physiologically weak and abnormal individuals, others produce individuals which in vigor, rate of growth, etc., surpass the normal. This phenomenon is perhaps more clearly and strikingly shown in pre-natal mortality (embryos dead in shell) than by any other character in fowls which can readily be measured. I hope shortly to publish a paper on this subject, and will only anticipate that paper here to the extent of saying that all the experience in this laboratory with cross-bred poultry agrees in showing that while there may be differences in the ease or success with which fertilization of the egg occurs in breed crosses,

these are relatively unimportant as compared with the differential embryonic mortality. The proportion of embryos which start to develop, but lack the power to complete development is uniformly much greater for some hereditary combinations than for others, regardless of the particular individuals used as parents, and under uniform conditions of incubation and of housing and feeding the parent stock.

It is in this direction that I am inclined to look for the explanation of the discordant results of ♂ 578's matings with barred F_1 females. The records give one the impression that the potency or absolute fecundity value of the several gametic factors had, because of the super-normal physiological condition, been bodily raised considerably above the normal for the strains used in these experiments. One cannot escape the feeling that all these birds were making higher records than individuals of the same gametic constitution but of more ordinary physiological character in general would have done. The scale of fecundity values has apparently shifted in an upward direction; in other words something similar to what occurs when two inbred strains of maize are crossed happened here.

Along this line is the only explanation for the outcome of these four matings that I am able to suggest. It is quite possible that it may have no bearing, and that the results are due to some peculiarity of gamete formation which can be suggested by some one. Personally, however, I am more inclined to keep to the solid ground of the observed physiological peculiarities of these matings rather than to 'juggle the genes.' Even in the hands of an adept the latter procedure runs some risk of taking one a great way from any solid ground of fact whatever.

Regarding these four matings 699-702 the following facts are definitely known:

1. High fertility of eggs.
2. Smallest embryonic (pre-natal) mortality of any particular gametic combination yet experienced in the work of this laboratory.
3. Great vigor and vitality of chicks at hatching and during growth.

4. Very low chick and adult mortality.

5. A higher egg production in practically all adult daughters than would be expected from the gametic constitutions *per se* of the parent forms, the latter being definitely known from their pedigrees and from their behavior (σ 578), or that of their full sisters in other types of matings.

I cannot escape the conviction that in some way the first four of these facts are connected with the explanation of the fifth. There the matter must be left for the present.

This case points to the importance of the physiological study of individuals in genetic work involving crossing. Only the most superficial aspects of this subject have ever been touched. The 'increased vegetative vigor' of first crosses is clear in some instances, but very far from being so in others, and nobody has ever shown by a clean-cut physiological investigation why or how the phenomenon occurs. Every breeder of experience knows that this is but one of many interesting and fundamentally significant physiological matters in connection with hybridizing and cross-breeding which need investigation. The animal breeder knows further that there are real objective phenomena, and not mere idle superstitions of the fancier at the basis of those things which the latter calls 'nicking' and 'prepotency,' for example. No doubt these things depend on simple genetic laws, but the point is that we do not now know scarcely anything definite (i.e., scientifically exact) about the phenomena, to say nothing of their underlying laws. The richness of the field which still remains quite unworked on the purely physiological side of genetics is, I think, only appreciated by the experienced breeder.

SUMMARY AND DISCUSSION OF RESULTS

The facts and their interpretation

In this paper is presented a detailed analysis and interpretation of a rather extensive series of data regarding the inheritance of fecundity in the domestic fowl. The basic data are derived from trap-nest records extending over a period of years. They include records from (a) pure Barred Plymouth Rocks; (b) Cor-

ish Indian Games; (c) the F_1 individuals obtained by reciprocally crossing these two breeds; and (d) the F_2 individuals obtained by mating the F_1 's *inter se* and back upon the parent forms in all possible combinations. The fully-pedigreed material made use of in this present paper includes something over a thousand adult females, each of which was trap-nested for at least one year, and many for a longer period. This material covers four generations. The birds of the fifth generation have just completed their winter records at the time of writing. Besides this fully pedigreed material, the collection and study of which has occupied five years there was available as a foundation, without which the results discussed in this page could not have been reached, nine years of continuous trap-nest records for Barred Plymouth Rocks, involving thousands of birds, which had been subjected during this long period to mass selection for increased egg production.

Altogether it may fairly be said that the material on which this paper is based is (a) large in amount, (b) extensive in character, and (c) in quality as accurate as it is humanly possible to get records of the egg production of fowls (Pearl 31). On these accounts the facts presented are worthy of careful consideration, and have a permanent value quite apart from any interpretation which may be put upon them.

The essential facts brought out in this study of fecundity appear to me to be the following:

1. The record of fecundity of a hen, taken by and of itself alone, gives no definite, reliable indication from which the probable egg production of her daughters may be predicted. Furthermore mass selection on the basis of the fecundity records of females alone, even though long continued and stringent in character, failed completely to produce any steady change in type in the direction of selection.

2. Fecundity must, however, be inherited since (a) there are widely distinct and permanent (under ordinary breeding) differences in respect of degree of fecundity between different standard breeds of fowls commonly kept by poultrymen, and (b) a study of pedigree records of poultry at once discovers pedigree lines (in some measure inbred of course) in each of which a definite, parti-

cular degree of fecundity constantly reappears generation after generation, the 'line' thus 'breeding true' in this particular. With all birds (in which such a phenomenon as that noted under *b* occurs) kept under the same general environmental conditions such a result can only mean that the character is in some manner inherited.

The facts set forth in paragraphs 1 and 2 have been presented, and, I believe, fully substantiated by clean-cut and extensive evidence, in previous papers from this laboratory. In the present paper it is further shown that:

3. The basis for observed variations in fecundity is not anatomical. The number of visible oöcytes on the ovary bears no definite or constant relation to the actually realized egg production.

4. This can only mean that observed differences (variations) in actual egg productions depend upon differences in the complex physiological mechanism concerned with the maturation of oöcytes and ovulation.

5. A study of winter egg production (taken for practical purposes as that from the beginning of the laying year in the early fall to March 1) proves that this is the best available measure of innate capacity in respect to fecundity, primarily because it represents the laying cycle in which the widest difference exists between birds of high fecundity and those of low fecundity.

6. It is found to be the case that birds fall into three well-defined classes in respect to winter egg production. These include (*a*) birds with high winter records, (*b*) birds with *low* winter records, and (*c*) birds which do not lay at all in the winter period (as defined above). The division point between *a* and *b* for the Barred Plymouth Rock stock used in these experiments falls at a production of about 30 eggs.

7. There is a definite segregation in the Mendelian sense of the female offspring in respect to these three fecundity divisions.

8. High fecundity may be inherited by daughters from their sire, independent of the dam. This is proved by the numerous cases presented in the body of this paper where the same proportion of daughters of high fecundity are produced by the same sire, whether he is mated with dams of low or of high fecundity.

9. High fecundity is not inherited by daughters from their dam. This is proved by a number of distinct and independent lines of evidence, of which the most important are: (a) continued selection of highly fecund dams does not alter in any way the mean egg production of the daughters (26, 27, 28, 30, 34, 35, 36, 37); (b) the proportion of highly fecund daughters is the same whether the dam is of high or of low fecundity, provided both are mated to the same male;¹⁶ (c) the daughters of a highly fecund dam may show either high fecundity or low fecundity, depending upon their sire; (d) the proportion of daughters of *low* fecundity is the same whether the dam is of high or of low fecundity provided both are mated to the same male.

10. A low degree of fecundity may be inherited by the daughters from either sire or dam or both.

11. The results respecting fecundity and its inheritance stated in paragraphs 3 to 10 inclusive are equally true for Barred Plymouth Rocks, Cornish Indian Games, and all cross-bred combinations of these breeds in F_1 and F_2 .¹⁷

The above statements are of definite facts, supported by a mass of evidence. Their truth is objective and depends in no way upon any theory of inheritance whatsoever. With this clearly in mind we may undertake their interpretation.

It is believed that these general facts, and the detailed results on which they are based, are completely accounted for and find their correct interpretation in the simple Mendelian hypothesis respecting the inheritance of fecundity in the fowl, which was outlined at the beginning of this paper and has been checked against the detailed data from each mating. This hypothesis involves the following points, each of which is supported by direct and pertinent evidence derived either from physiological and statistical

¹⁶ This is true, of course, only for certain gametic types of low fecundity females, as will be clear to anyone who has studied the detailed evidence. This limitation, however, in no wise diminishes the force of this particular evidence in favor of the conclusion standing at the beginning of paragraph 9.

¹⁷ And F_3 . It has not been thought wise to delay publication of this paper any longer in order to include the data for F_3 . It may be said however that they are in full accord with those which have been obtained from earlier cross-bred generations and the parent forms.

studies of fecundity, or from the detailed data respecting the mode of inheritance of this character.

It is assumed in this hypothesis that:

1. There are three distinct and separately inherited factors upon which fecundity in the female fowl depends.

2. The first of these factors (which may be called the anatomical) determines the presence of an ovary, the primary organ of the female sex. The letter *F* is used throughout to denote the presence of this factor.

3. There are two physiological factors. The first of these (denoted by L_1) is the basic physiological factor, which when present alone in a zygote with *F* brings about a low degree of fecundity (winter record under 30 eggs). This factor is under no limitations in gametogenesis but may be carried in any gamete, regardless of what other factors may be also present.

4. The second physiological factor (denoted by L_2) when present in a zygote together with *F* and L_1 leads to a *high* degree of fecundity (winter record over 30 eggs). When L_1 is absent, however, and L_2 is present the zygote exhibits the same general degree of fecundity (under 30) which it would if L_1 were present alone. These two independent factors L_1 and L_2 must be present together to cause high fecundity, either of them alone, whether present in one or two 'doses,' causing the same degree of low fecundity.

5. The second physiological factor L_2 behaves as a sex-limited (sex-correlated or sex-linked) character, in gametogenesis, according to the following rule: the factor L_2 is never borne in any gamete which also carries *F*. That is to say, all females which bear L_2 are heterozygous with reference to it. Any female may be either homozygous or heterozygous with respect to L_1 . Any male may be either homozygous or heterozygous with reference to either L_1 , L_2 or both.

How well this hypothesis agrees with the facts has been shown in detail in the preceding sections. By way of summary the following table shows the accord between observation and expectation for all matings of each general type taken together. For reasons set forth below, the lumped figures do not give an alto-

TABLE 33

showing the observed and expected distributions of winter egg production for all matings taken together

MATING	WINTER PRODUCTION OF DAUGHTERS			
	Class	Over 30	Under 30	Zero
All B.P.R. \times B.P.R.....	Observed..	365½	259½	31
	Expected ..	381.45	257.45	17.30
All C.I.G. \times C.I.G.....	Observed..	2	23	15
	Expected ..	0	25	15
All F_1	Observed..	36	79	8
	Expected ..	26.5	86.75	9.75
All F_2 and back-crosses ¹ ...	Observed..	57½	98½	23
	Expected...	68.60	95.00	15.40

¹ With exception of the matings of C.I.G. σ 578 \times Barred F_1 ϕ ϕ . Cf. p. 246.

gether fair estimate of the matter, but some sort of a summary is necessary.

Considering the nature of the material and the character dealt with it can only be concluded that the agreement between observation and hypothesis is as close as could reasonably be expected. The chief point in regard to which there is a discrepancy is in the tendency, particularly noticeable in the B.P.R. \times B.P.R. and the F_2 matings, for the observations to be in defect in the 'Over 30' class and in excess in the 'Zero' class. The explanation of this is undoubtedly, as has been pointed out in the body of the paper, to be found in disturbing physiological factors. The high producing hen, somewhat like the race horse, is a rather finely strung, delicate mechanism, which can be easily upset, and prevented from giving full normal expression to its inherited capacity in respect to fecundity.

In order to forestall any possible change of manipulation of the data to support a particular hypothesis all of the figures (with the exception of 7 birds discussed on p. 233 and the F_2 mating of σ 578) have been entered throughout exactly as they stood on the original books of record. That is to say, some birds known to be physiologically abnormal or pathological have not been rejected, but have been entered in the tables and then discussed in

the accompanying text. Whether this is accounted a justifiable procedure or not will depend upon one's point of view in some degree. The investigator is usually expected to reject abnormal material. But in view of the rather hysterical attacks upon geneticists and their method of work now becoming so fashionable in this country, if for no other reason, it seems best to follow the plan of publishing all the data. The opponents to the views which underlie the Mendelian interpretation here advanced are quite welcome to make as much capital as they are able to out of the discrepancies between observation and theory in the several tables. It seems only fair, however, to ask that a judgment of the adequacy of the hypothesis be not formed from this summary table 33, but instead from the detailed data in the body of the paper.

Possible criticisms

In consideration of the fact that this paper constitutes one of the first attempts to apply a Mendelian interpretation to the facts regarding the inheritance of an economically productive character of an animal, and in view of the possible application of the results or the methods of this paper to other productive characters of other organisms it is important to examine carefully and critically the nature of the evidence and the objections which may be brought against the conclusions. In the first place it is important to note once more that the data and their interpretation are kept separate throughout, and that the value of the former is not lessened if the latter is later found to be completely invalid, or in need of modification. It is scarcely necessary to say that the Mendelian hypothesis here presented is the only simple one which the writer has been able to discover, after over two years of study directed (whenever the time was available) towards this particular end, which is capable of accounting satisfactorily for all the facts. Very many other Mendelian schemes for the inheritance of fecundity have been tested against the facts in the course of the work and discarded, one by one, because inadequate. Of course, it still remains quite possible, though perhaps not very probable, that there may be an even simpler hypothesis which

will equally well or better account for the facts. If so, by all means let us have it. But in the meantime, it may be fairly be said, that the hypothesis here presented brings together under a few, symbolically simple, general statements a wide range of very diverse and complex facts of inheritance.

The strongest general evidence that the Mendelian hypothesis here presented is at least a close approximation to the truth in respect to the inheritance of fecundity in the fowl is found in the fact that it accounts equally well for so wide a range of diverse phenomena. In the two 'pure' parent races, one of generally high and the other of generally low fecundity; the two reciprocal crosses; and the twelve different kinds of matings in F_2 , we have a series of really independent measures of the validity of the hypothesis. It accords with the facts in all but one (the matings of C.I.C. ♂ 578 with Barred F_1 ♀ ♀) of all of the different types of matings tested. The one exception probably has a physiological explanation (pp. 246-250). In view of these facts the cumulative probability that the hypothesis applied represents at least a reasonable approximation to the true interpretation of the results becomes very great.

A possible criticism of the whole method of this investigation might be found in reference to the measure of fecundity which has been used throughout, namely, the winter egg production. Regarding this matter it should be said that the very reason why winter egg production was adopted as the unit of measure in all of the fecundity work of this laboratory was because a thorough biometrical and physiological study of egg production in fowls showed beyond question that winter production was the best practicable index or measure of a fowl's innate or constitutional capacity in respect of fecundity. The reasons for this conclusion have been set forth in this and former papers from the laboratory and need not be repeated *in extenso* here. The most significant of them is that the differences in observed production between individuals of different innate fecundity capacities are relatively greater in respect of winter productions than of any other time unit that can practically be employed in the measuring of this character. To suppose, however, that the results set forth in this

paper depend for their existence upon the use of this particular time period of production as a measure of fecundity has no warrant in fact. Precisely the same results (in principle) would be obtained if yearly production records were used in the analysis. During the whole of this work complete yearly records have been kept and have been studied. They show in every essential particular the same kind of results as those of this paper. There are objections to the use of the year as a unit of measure, however, which may not be obvious to one inexperienced in these matters. In the first place, it is very much more difficult to keep large flocks of hens in normal, and healthy physiological condition over a whole year period than over a shorter period. Again the risk of an accident (say the use of bad feed or something of the kind occurring and upsetting the birds physiologically, and coincidentally rendering their fecundity records abnormal and in greater or less degree useless, is increased just in proportion as the time unit is increased. Further the year period includes as a too dominant feature, the spring egg production. The production during the months of March, April and May is practically worthless (and has long been so recognized by experienced poultrymen) as an index or measure of the true, innate or constitutional fecundity capacity of the individual. During these months (in northern latitudes) all hens which are not diseased, malformed, infantile or senile, lay anywhere from 'well' to 'very well.' There is relatively little difference between the most and the least fecund at this season. This period is therefore worthless as a measure of fecundity, and its inclusion in any longer period makes that by so much the less valuable as a measure.

In view of all these considerations it seems certain that the results obtained are not open to criticism on the ground of the time unit used as a measure of fecundity.

Another matter which needs careful consideration is as to the possibility of unconscious bias having influenced the results themselves. In other words, to what extent does the personal equation factor enter into this fecundity work? It can be fairly said. I think, that there is less opportunity for unconscious bias to affect the results here than in genetic work on most other charac-

ters. The reason is because of the impersonal and objective character of the original records in the case of fecundity. The original trap-nest records on which this whole study is based were made by Mr. F. Walter Anderson. He had neither knowledge of, nor interest in, the use of which any particular record or set of records were to be put. He was solely concerned to make as accurate record as possible of the laying of each individual hen. The system of record taking used is such that it was impossible for him to have any notion of what the total production of any given bird up to a particular date had been. The chance for bias or personal equation influencing results is excluded when, as in the present case, one person makes the basic records, and has nothing whatever to do with their analysis, while another person analyzes the data but has nothing directly to do with their collection.

Another safeguard on the results in this same direction, and also in another, is found in the fact that birds belonging to the same family (full sisters) were not given identifying numbers which would make it possible to be certain or even to surmise that they were sisters, without consultation of the pedigree records. The numbering of the birds for identification each year was purely at random and without any regard whatsoever to relationship. Furthermore members of the same family were distributed at random through the different pens and houses. No attempt is ever made, from the day the chicks hatch, to keep the birds from one family together. Indeed it is important that they be scattered at random through the flock in order to insure uniformity of average environmental conditions.

The writer has no desire to generalize more widely from the facts set forth in this paper than the actual material experimentally studied warrants. It must be recognized as possible, if not indeed probable, that other races or breeds of poultry than those used in the present experiments may show a somewhat different scheme of inheritance of fecundity. The directions in which deviations from the plan here found to obtain may, at least *a priori*, most probably be expected are two. These are: (a) differences in different breeds in respect to the absolute fecundity value or

worth of the factors which determine the expression of this character, and (b) gametic schemes which differ from those here found either in the direction of more or fewer distinct factors being concerned in the determination of fecundity, or in following a totally different type of germinal reactions.

Regarding the first point it will be recalled that in several places in the body of the paper it has been suggested that the absolute fecundity value (i.e., the degree of actual fecundity determined by the presence of the gametic factor) may differ for the factor L_1 in the case of the Barred Rock as compared with the Cornish Indian Game breed. It is hoped later to take up a detailed study of this point, on the basis of the material here presented, and additional data now in process of collection. Wherever there is a difference in the absolute fecundity value of the L_1 factor, it means that the division point for the classification of winter productions should be taken at a point to correspond with the physiological facts. In this first study the division at 30 eggs has been found to accord sufficiently well for practical purposes with the actual facts. Similarly the absolute fecundity value of the excess production factor L_2 may be different in different breeds. In applying the results of this paper to the production statistics of other breeds of poultry the possibility of differences of the kind here suggested must always be kept in mind.

The second point (the possibility of gametic schemes for fecundity differing qualitatively from that found in the present study) is one on which it is idle to speculate in advance of definite investigations. I wish only to emphasize that nothing is further from my desire or intention than to assert before such investigations have been made that the results of the present study apply unmodified to all races of domestic poultry.

It cannot justly be urged against the conclusions of this study that the Mendelian hypothesis advanced to account for the results is so complicated, and involves the assumption of so many factors or such complex interactions and limitations of factors, as to lose all significance. As a matter of fact the whole Mendelian interpretation here set forth is an extremely simple one, involving essentially but two factors. This surely does not

indicate excessive complication. To speak in mathematical terms, by way of illustration merely, it may fairly be said, that the formula here used to 'fit' the data, has essentially the character of a true graduation formula, rather than that of an interpolation formula. The number of constants (here factors) in the formula is certainly much less than the number of ordinates to be graduated.

There is no assumption made in the present Mendelian interpretation which has not been fully demonstrated by experimental work to hold in other cases. That the expression of a character may be caused by the coincident presence of two (or more) separate factors, either of which alone is unable to bring it about, has been shown for both plants¹⁸ and animals by a whole series of studies in this field of biology during the last decade. To find examples one has only to turn to the standard hand-books summarizing Mendelian work, as for example those of Bateson and Baur. Again sex-linkage or correlation of characters in inheritance has been conclusively demonstrated for several characters in fowls by the careful and thorough experiments of a number of independent investigators. Finally it is to be noted that Bateson and Punnett (4) have recently shown that the inheritance of the peculiar pigmentation characteristic of the silky fowl follows a scheme which in its essentials is very similar to that here worked out for fecundity.

The selection problem

The results of the present investigation have an interesting and significant bearing on the earlier selection experiments on fecundity at this Station. It is now quite plain that continued selection of highly fecund females alone could not even be expected to produce a definite and steady increase in average flock production. The gametic constitution of the male (in respect especially to the L_2 factor) plays so important a part in determining the fecundity of the daughters that any scheme of selection which

¹⁸ Particularly important here are the brilliant researches of Nilsson-Ehle (24, 25) on cereals, and of Baur (2) on *Antirrhinum*.

left this out of account was really not 'systematic' at all but rather almost altogether haphazard. It has been repeatedly shown in the body of the paper that the same proportion of daughters of high fecundity may be obtained from certain mothers of low fecundity as can from those of high fecundity, provided both sets of mothers are mated to males of the same gametic constitution. What gain is to be expected to accrue from selecting high laying mothers under such circumstances, at least so far as concerns the daughters?

'Selection' to the breeder means really a system of breeding. 'Like produces like,' and 'breed the best to get the best,' these epitomize the selection doctrine of breeding. It is the simplest system conceivable. But its success as a system depends upon the existence of an equal simplicity of the phenomena of inheritance. If the mating of two animals somatically a little larger than the average always got offspring somatically a little larger than the average, breeding would certainly offer the royal road to riches. But if, as a matter of fact, as in the present case, a character is not inherited in accordance with this beautiful and childishly simple scheme, but instead is inherited in accordance with an absolutely different plan, which is of such a nature that the application of the simple selection system of breeding could not possibly have any direct effect, it would seem idle to continue to insist that the prolonged application of that system is bound to result in improvement.

It seems to me that it must be recognized frankly that whether or not continued selection of somatic variations can be expected to produce an effect on the race depends entirely on the mode of inheritance of the character selected. In other words, any systematic plan for the improvement of a race by breeding must be based and operated on a knowledge of the gametic condition and behavior of the character in which improvement is sought rather than the somatic. Continued mass selection of somatic variations as a system of breeding, in contrast to an intelligent plan based on a knowledge of the gametic basis of a character and how it is inherited, seems to me to be very much in the same case as a man who, finding himself imprisoned in a dungeon with a securely locked and very heavy and strong door with the

key on the inside, proceeded to attempt to get out by beating and kicking against the door in blind fury, rather than take the trouble to find the location of the key and unlock the door. There is just a possibility that he could finally get out in a very few instances by the first method, but even in those cases he would be regarded by sensible men as rather a fool for his pains.

Of course what has been said is not meant to imply that selection on the basis of somatic conditions may not have a part in a well considered system of breeding for a particular end. In many cases it certainly will have. Thus in the case of fecundity in the fowls, selection of mothers on the basis of fecundity records is essential in getting male birds homozygous with respect to L_1 and L_2 . But the point which seems particularly clear in the light of the present results is that blind mass selection, on the basis of somatic characters only is essentially a haphazard system of breeding which may or may not be successful in changing the type in a particular case. There is nothing in the method *per se* which insures such success, though that there is inherent potency in the method *per se* is precisely the burden of a very great proportion of the teaching of breeding (in whatever form that teaching is done) at the present time.

It seems to me that it has never been demonstrated, up to the present time, that continued selection can do anything more than:

1. Isolate pure biotypes from a mixed population, which contains individuals of different hereditary constitution in respect to the character or characters considered.

2. Bring about, as a part of a logical system of breeding for a particular end, certain combinations of hereditary factors which would never (or very rarely) have occurred in the absence of such systematic selection; which combinations give rise to somatic types which may be quite different from the original types. In this way a real evolutionary change (i.e., the formation of a race of qualitatively different hereditary constitution from anything existing before) may be brought about. This can unquestionably be done for fecundity in the domestic fowl. But here 'selection' is simply one part of a system of breeding, which to be successful must be based on a definite knowledge of gametic as well

as somatic conditions. It is very, very far removed from a blind 'breeding of the best to the best to get the best.' The latter plan alone may, as in the case of fecundity, fail absolutely to bring about any progressive change whatever.

It has never yet been demonstrated, so far as I know, that the absolute somatic value of a particular hereditary factor or determinant (i.e., its power to cause a quantitatively definite degree of somatic development of a character) can be changed by selection on a somatic basis, however long continued. To determine, by critical experiments which shall exclude beyond doubt or question such effects of selection as those noted under 1 and 2 above, whether the absolute somatic value of factors may be changed by selection, or in any other way, is one of the fundamental problems of genetics.

Prepotency

One of the least understood phenomena in genetics is that which the practical breeder calls 'prepotency.' When the scientific student of genetics deals with the matter at all he is rather apt either to throw it over entirely as a 'breeder's superstition,' or to take it as something 'given' to help him out of a difficulty in the interpretation of results which fail to conform to expectation. Some time a more searching investigation of this phenomenon must be made than is implied in either of these lines of procedure.

In a former paper (27, p. 324), it was suggested that the evidence indicated, for certain productive characters at least, that hereditary high performance tended to behave as a Mendelian dominant to hereditary low performance. The following statement was then made:

If this suggestion is true it gives at once, I think, a possible clue to the explanation of a part at least of the known facts regarding what is called prepotency in the practical breeding of domestic animals for performance. It is customary in practice to regard an animal as prepotent in breeding for performance when the progeny of that individual uniformly tend to resemble it closely in respect to the character bred for, regardless of the other parent in each mating. Let it now only be considered that the great sire, say, of speed or of milk production belongs to a line having a

high genotype with regard to those characters; then it is to be expected, on the hypothesis under consideration, that his progeny will tend on the average to be like himself in performance regardless of what he is mated with, because any female to which he is mated will be either of a high genotype like himself or of a lower one. But if genotypic high performance is dominant over genotypic lower performance, then all the offspring in the first generation must approximate to the high condition exemplified in the sire. But this is the very essence of what is called prepotency in actual breeding practice.

It seems to me that certain of the facts set forth in this paper give strong support to this view. A class 1 B.P.R. ♂ (= fL_1L_2 . fL_1L_2) will get all high producing daughters (barring physiological defects of development) regardless of the females to which he is mated. He will show all the objective phenomena of 'prepotency.' B.P.R. ♂ 550 is an example of this. A class 7 B.P.R. male would, in breeders' parlance, be regarded as less prepotent than a class 1 male, but, even so, more prepotent than the general run of the flock.

The essential point here should not be misunderstood. It is not, of course, contended that simple Mendelian 'dominance' in general, and prepotency are the same thing. More than that is demanded. It is only suggested that a homozygous dominant individual, when high performance is dominant over low, has all the objective characteristics of a prepotent individual in the breeder's sense.

That this suggestion explains all the facts regarding prepotence is by no means asserted. It seems to me, however, that it does furnish the explanation for a part of the phenomena at least, and by so much helps towards a final solution, since it brings us nearer to the kernel of the problem.

The practical bearing of these results

To the practical poultryman the data and conclusions of this paper would appear to have some significance. They make it possible to outline a scheme of breeding for increased egg production which shall be intelligently directed towards the attainment of that end. This, however, is not the place to discuss such a scheme. That will be undertaken later in another place.

In bringing this long piece of work to a close I desire to express my deep obligation and gratitude to those who have aided in the carrying out of the investigation. All of raw data (the trap-nest records) were made by Mr. Frederick Walter Anderson, and checked and copied by Mrs. Lottie McPheters Maxwell. Lacking the stimulus of scientific interest in the outcome, these two assistants have collected and handled these data with never-failing fidelity to the highest ideals of scientific accuracy. Such unswerving loyalty to the Station, the Biological Laboratory, and the investigation itself as they have shown is worthy of the highest praise.

Finally, my greatest debt is to my good friend Dr. Charles D. Woods, the Director of this Station. Without his loyal support in every possible way, his ever-ready encouragement, and his far-sighted and broad-minded appreciation of the spirit and meaning of scientific research, this investigation could not have been carried out. He it was who laid the basic plans fourteen years ago for the egg-production studies of which the present investigation is the outcome. His was the faith which put at the disposal of the work greatly increased financial and material support at the very time when the outlook for any significant practical success from the experiments seemed darkest. It would indeed be a fortunate thing if such a broad and thoroughly and purely scientific spirit was more generally to be found in executive control of agricultural research in this country.

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AN EXPERIMENTAL ANALYSIS OF THE RELATION BETWEEN PHYSIOLOGICAL STATES AND RHEOTAXIS IN ISOPODA

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I. INTRODUCTION

This paper is the result of an investigation into the rôle played by the 'physiological state' of the organism in determining its reactions. Jennings ('06, pp. 286-292) summed up the work along this line, by Moebius ('73), Romanes ('85), Preyer ('86), Uexküll ('97, '99, '00.) Yerkes ('02), Smith ('02), Pearl ('03), Mast ('03), Bohn ('03, '03 a, '05), Gamble and Keeble ('03), Yerkes and Huggins ('03), Jennings ('04 b, '04d), Spaulding ('04), Holmes ('05) and Harper ('05). From this work he drew the following conclusions: (1) Changes in activity depend on changes in physiological states. (2) Reactions to external conditions depend on physiological states. (3) The physiological state may be changed by (a) progressive internal processes, (b) by the action of external agents, (c) by the activity of the organism. (4) External agents cause reactions by changing the physiological state of the organism. (5) The behavior of the organism at any moment depends on its physiological state at that moment. (6) Physiological states change either according to the laws affecting metabolism or according to those controlling stimulation.

These statements, however, were supported by little direct experimental evidence and Jennings recognized this, for he says ('06, p. 251):

The diverse physiological states of lower organisms have been little studied. This is partly because it is rarely possible to observe them directly; it is only through their effects upon action that they become evident. Thus the real data of observations are the actions; if we con-

sider these alone, we could only state that a given organism reacts under the same external conditions sometimes in one way, sometimes in another. This would give us nothing on which to base a formulation and analysis of behavior, so that we are compelled to assume the existence of changing internal states. This assumption besides being logically necessary, is of course, supported by much positive evidence drawn from diverse fields; and *there is reason to believe that in time we shall be able to study these states directly.*¹ Before we can come to a full understanding of behavior, we shall have to subject the physiological states of organisms to a detailed study and analysis, as to their objective nature, causes and effects.

In commenting on Jennings' position, Mast ('11, pp. 375-378) placed some emphasis on another factor. He said in part:

It is evident, that while there is some evidence bearing on physiological states we know but little about their nature and regulation. For all that is known to the contrary, subjective factors, entelechies, or psychoids, factors foreign to inorganics, may have a hand in controlling physiological changes and consequently the reactions. Whether or not there are any such phenomena is the question at issue. . . . But until this question is settled . . . those who maintain . . . there are no entelechies are certainly no more scientific than those who maintain the opposite.

The analysis of the relationship existing between the physiological state of the isopods used and their rheotactic reaction has been carried on in accordance with the statements of Jennings just quoted. The work has proceeded far enough at present to enable one to predict with certainty the action that will result from a physiological state experimentally produced, so that in this case the behavior of the animals becomes a check on the production of a physiological state as well as an indicator that a change must have occurred. While it is as yet impossible to control with certainty all the minor details of the reaction, yet sufficiently complete control has been maintained to show that in the rheotactic response with these animals there is no necessity to call in any 'factor foreign to inorganics' in order to explain the changes in physiological states.

¹ Italics mine.

1. MATERIAL

The isopods used in this study have almost all been *Asellus communis* Say. In a very few cases trials have been made with *Manecasellus danielsii* Rich. *Asellus communis* is the common fresh water isopod. Richardson ('05, p. 420) gives its distribution as extending from Massachusetts and Connecticut on the east to Illinois and Louisiana on the west. Near Chicago it is found throughout the year in the older ponds of the series at the south end of Lake Michigan (Allee, '11, p. 126; Shelford '11 a, maps) and in young streams, particularly those which have permanent pools and temporary riffles. Most of the stream isopods used in the course of this work were taken from the County Line Creek near Glencoe, Illinois (Shelford '11, maps pp. 14; 17). In general their stream distribution parallels that of the horned dace, *Semotilus atromaculatus* (Shelford '11, p. 17). In the spring these isopods are very abundant in the small temporary ponds especially where there is a thick covering of leaves over the bottom which guards against too severe desiccation in the dry periods. The *Manecasellus danielsii* have never been taken from a stream or from the small summer-dry ponds in this vicinity. They are limited mainly to the series of ponds mentioned above, although some have been taken from a spring-fed, watercress marsh at Cary, Illinois. In the Chicago area they have never been found in a place not containing *A. communis*.

In early spring the isopods are usually found along the margins of the ponds, later in the season they are more common in deeper water (Allee, '11). They crawl around over the vegetation and bottom and are almost never seen swimming. There is a distinct daily movement that is more pronounced in the deeper water. Here they are more numerous at the surface during times of dim light and retire to the bottom when exposed to bright sunlight. If the pond dries, they burrow into the mud and are thus able to withstand droughts extending over several months. In the streams, they are usually found in protected positions, often hiding among a bunch of leaves or other debris. In streams with rocky beds they may occur under stones.

Much material collected from all places isopods are known to inhabit in this region, was identified by Miss Richardson in 1910 as consisting entirely of the two species mentioned. The appearance of the two is entirely different, so that there is no chance of any of the results obtained being due to a mixing of species. Regarding *A. communis*, Miss Richardson says, in a private communication:

Asellus communis is a very variable species. The uropods in some specimens differ considerably from the typical form, in being shorter and of varying lengths, while in other specimens they are narrower. I think these differences may be due to size, age, and in some cases the parts may be in process of regeneration. Then too the propodus of the first pair of legs in the male differs in the specimens, being larger in some than in others and with spines more pronounced. There are so many intermediate stages that it was not possible to group the specimens into varieties as I had at first supposed could be done.

A careful study of a large number of specimens from both ponds and streams showed these variations to be equally common in all habitats. Thus the differences found in the reactions cannot be due to taxonomically differing races.

2. GENERAL REACTIONS

The experimental work upon which this paper is based was begun in the summer of 1909 and has been in progress continuously since that time. The early experiments upon the general responses run parallel with those of Banta ('10). A summary of these general reactions of *A. communis* will be given, in order that the conditions of the later experiments may be better appreciated. Unless otherwise indicated, the results are my own and in almost every instance they support the results obtained by Banta.

1. The main breeding period in these isopods extends from the middle of March to the middle of July but this may be extended in scattered cases until the beginning of cold weather. During the copulation the males carry the females for as many as three days. The developmental period is about three weeks and on the average forty offspring are liberated each time. The number of offspring may be much larger, and in one instance two hun-

dred young were taken from a single brood pouch. More than one brood is brought forth by the same female in one season. Some of the animals may live over another winter although many of them die off at the close of the breeding season. In nature the breeding habitat is restricted to the most favorable locations. Banta did no work with animals during the breeding season.

2. The food of *A. communis* as determined both by observing their feeding habits and by examining their alimentary tract, consists of algae, larger green water plants, protozoa, decaying leaves and dead animals.

3. They are strongly positive to gravity and water pressure when these are acting alone but if light is introduced the reaction is controlled by the light.

4. *A. communis* is strongly positive to tactile stimuli. The hairs are sensitive to touch so that a response is given, even when antennae, antennules, and uropods are removed. The positive thigmotaxis is shown by their tendency to collect in corners or under thin mica plates. Again if subjected to the action of light they will disregard their thigmotactic optimum and respond to light alone.

5. Their temperature optimum varies, depending on the temperature in which they have been kept. Sudden temperature changes in either direction cause them to collect in bunches, more extreme changes cause the 'pill bug' reaction and if these conditions continue death results.

6. *A. communis* is negative to direct sunlight or to a large amount of diffuse light although they are positive to room light admitted through a very small opening. Young animals are negative to all light intensities used. The adults have a light optimum, as this shifts they change their position until they are in optimum conditions. The response to light is affected by their previous exposure. Banta ('10, pp. 263-269) found in this connection that after being in darkness for several hours *Asellus* is positive to all light intensities tried, the duration of the positive response depending on the intensity of the light. In my experiments however they were most positive to faint light after forty hours continuous exposure to a light intensity of 80 candle

eters. If exposed to the colors of the spectrum they collect in the red end and animals exposed to red light act almost as if in complete darkness. Aselli are nocturnal in their habits and in ponds or aquaria there is a diurnal movement, depending, in part at least, on light conditions.

7. Banta (l. c., pp. 440-467) found that they were sensitive to mechanical stimulation with bristles; with localized currents of water; with concussion; and with vibrations at the rate of 100 per second. The results with localized currents of water showed the most sensitive parts of Asellus to be on the head and at the base of the antennae, and these responses were only gained with the strongest currents used.

3. METHODS

At the beginning of the work on rheotaxis it was necessary to devise some method that would permit rapid testing of a large number of animals and in which the personal equation should be reduced to a minimum. Also the method used must be applicable to both field and laboratory work. For this reason it was thought best to use a circular current, although Allen ('10) in an unpublished master's thesis showed that the current set up in a circular pan is not straight but forms a diverging spiral.

The method used is as follows: The isopods were placed in an enamel-ware pan 25 cm. in diameter and 6 cm. deep. In order that they might have a firm foot hold for crawling the pan bottom was covered with a layer of bees-wax. The animals were placed in 2 cm. of the same water in which they had been kept. In the laboratory the pan was then set in a dark box under an illumination of one candle meter. One side of this box was curtained so that later the tests could be made without introducing outside light. Usually five isopods were used although this number was varied with the size and condition of the animals. The animals were undisturbed for fifteen minutes in order to allow them to become accustomed to the new conditions and to permit a recovery from the shock of handling. In case they had been kept at a temperature differing from that of the room the pan was

placed in a bath that would keep the temperature within one degree of that to which they were accustomed. In the field the trials were made in diffuse light, with all other conditions as near those of the laboratory trials as was possible.

After fifteen minutes a current was produced by stirring with a glass rod about 8 mm. in diameter. In order to secure as even a current as possible the rod was run five times around the pan at a distance of about 4 cm. from the edge and at a uniform rate. The attempt was made to keep from stirring the animals from the bottom. *Usually they remained along the edge of the pan and thus were in that part of the current that shows the least spiral tendency.* Time was taken with a stop watch for one minute after the stirring stopped and the reaction of each animal for the greater part of the minute was recorded. Thus if an animal went against the current for forty seconds and with it for the remainder of the minute it was counted positive, while if it went with the current half the time and against it the other half it was counted indifferent. At the end of the minute reaction, the current was set up in the *reverse direction*. The reason for reversing the direction of the current, is that isopods tend to continue in the same direction in which they are started. Thus if by accident negative isopods are all going against the current and at the end of the minute's reaction the current is merely renewed, there would be a tendency to remain positive although in reality their normal reaction would be negative or indifferent. If on the other hand the current is reversed, then if they are strongly positive they will reverse their direction thereby showing that they are reacting to the direction of the current and not to chance factors.

Trials were continued in this fashion until ten consecutive tests had been made. These results were recorded and the percentage of animals going positively, negatively, and indifferently was calculated. It is to be regretted that there is as much left to the personal equation as there is in this method, yet it furnishes a fairly stereotyped set of trials that have given closely comparable results. The positive reaction obtained under these conditions consists of two factors, namely: (1) The percentage of positive responses numerically stated and (2) the positiveness with which

the response may be given, that is, the speed and definiteness of the response. Experiments have shown that these two qualities of the response are closely correlated and when either is stated in this discussion, the other is always implied.

The possibilities of this method are shown in table 1. The Aselli used in this experiment were carefully selected from a stock of stream isopods that had been reared in known conditions in the laboratory. They were about five months old and were between 9 and 10 mm. in length. All were in the same stage

TABLE 1

Table showing possibilities of the method used. Eight stream Aselli, 8 to 9 mm. long; twenty-four hour intervals

O ₂ cc. per liter	RESPONSE*			NUMBER OF TRIALS	TEMPERATURE (°C)
	per cent +	per cent -	per cent α		
6.49	90	10	0	80	18
6.49	89	11	0	80	18
7.11	89	10	1	80	18
6.90	91	9	0	80	18
6.90	85	11	4	80	18
7.00	91	9	0	80	18
7.40	90	8	2	80	15

Average positive response 89.4 per cent.

Greatest deviation from average 4.9 per cent.

*+ indicates a positive reaction.

- indicates a negative reaction.

α indicates an indifferent reaction.

regarding moulting, and were entirely normal in every way. Throughout their life they had been kept in still water having an average of about 6 cc. of oxygen per liter.

From table 1 it will be seen that with animals in approximately the same physiological state, the experimental error of the method used is almost 5 per cent. This error is too large for purely quantitative results, but it will not interfere greatly with the comparisons that are to be made in this work. In no case however is any importance attached to experimental results that do not show a difference of at least 20 per cent, so that the possible error of 5 per cent cannot affect the conclusions drawn.

The results of another check on the constancy of the rheotactic response to a circular current are given in figure 1, which shows graphs of twenty-five successive responses of one male *Asellus*. The animal used was a stream isopod 11 mm. long, that had been

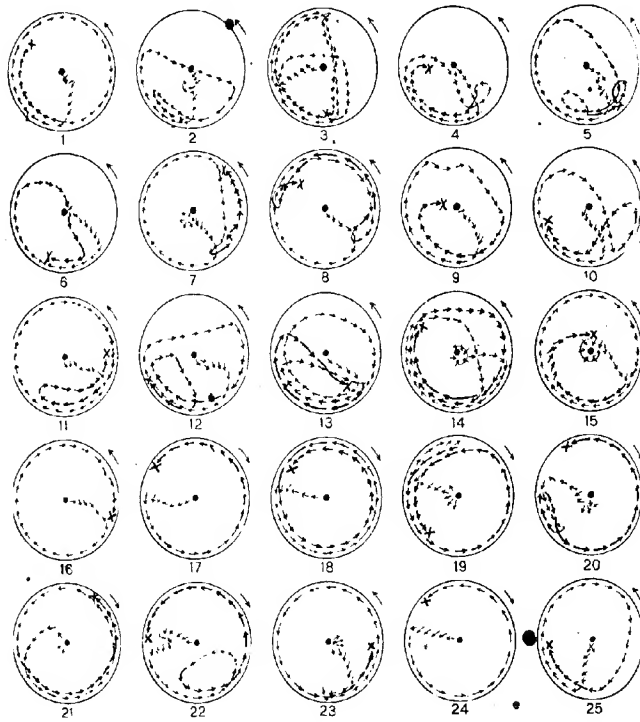


Fig. 1 Twenty-five successive rheotactic reactions of one stream *Asellus*

kept in water with the oxygen at air saturation. In this case the trials were made in a glass dish 10 cm. in diameter. The current used was strong enough to sweep the isopod to the center each time and the presence of the spiral current is plainly shown by the path taken in reaching the circumference of the dish.

In the figure the starting point is indicated by a large dot; the end point, by a cross. The arrow on the outside of the circle indicates the direction of the current.

In all cases, excepting no. 23, the animal responded positively to the current, that is, it gave a 96 per cent positive response. Evidently its normal reaction was a positive one and the one failure to go against the current would then mean a chance turning that was not corrected. That is, in this case there is an experimental error of 4 per cent, which checks well with that shown in table 1. These results also show that the isopods will give their normal response for at least twenty-five successive trials, so that it is entirely safe to take the first ten responses as indicating the normal behavior of the animals tested.

During these experiments the oxygen content of the water has been determined by the Winkler method. During the first part of the work, the method was followed as outlined in the report of the committee on standard methods of water analysis to the laboratory section of the American Health Association ('05, pp. 74-77). The free carbon dioxide was determined by direct titration with N/22 sodium carbonate using phenolphthalein as an indicator. This method is described in the same report (pp. 72-73). After the appearance of Birge and Juday's work ('11, pp. 13-24), their methods were followed wherever the technique appeared better. Birge and Juday compared this method of determining the oxygen content of the water with that of boiling and found (*l. c.*, pp. 11-12) that the amount of variation in results from the two methods was not more than was the case in duplicate determinations by the same method. V. E. Shelford and the writer verified these results and the methods used have proved eminently satisfactory for rapid biological work.

II. RHEOTACTIC RESPONSE UNDER NATURAL CONDITIONS

• STREAM ASELLI

a. Normal adults

Table 1 and figure 1 show responses of stream isopods at their highest rate of positiveness. Usually under the conditions used the per cent of positive response was somewhat lower. A list of the results of these tests is given in table 2. It will be seen that the results of different trials vary somewhat, the greatest varia-

TABLE 2
Normal rheotactic response of adult stream Aselli

O ₂ cc. per liter	RESPONSE			NUMBER OF TRIALS	TEMPERATURE C
	per cent +	per cent -	per cent x		
5.12	72	20	8	60	17
5.46	78	12	10	50	17
5.5	70	12	18	50	18
5.69	78	12	10	50	18
5.92	70	24	6	80	18
5.97	70	18	12	60	17
6.03	80	20	0	30	18
6.03	88	6	6	60	18
6.15	76	14	10	80	18
6.26	80	14	6	50	20
6.36	75	25	0	40	17
6.49	89	8	3	60	18
6.55	93	7	0	80	12
6.57	72	18	10	50	19
6.77	86	13	1	70	15
7.00	88	6	6	50	23
7.02	72	8	20	50	13
7.51	82	17	1	80	10
7.54	73	27	0	30	18
8.14	79	21	0	80	18
8.14	85	11	4	80	17
8.14	83	12	5	80	17
8.59	86	4	10	50	16
9.22	84	4	12	50	6
9.31	78	8	14	50	6
9.34	74	10	16	50	8
10.25	78	18	4	50	11
6.41	79	14	7	1570	

tion being 18 per cent from the mean. These variations may be due to length of time since moulting or age. The group giving the highest positive response had been carefully selected to represent animals in the best possible condition. In all the other cases the animals were simply picked at random from the general stock. The variation in oxygen content of the water within the limits given in table 2 does not appear to have any marked significance although in general the lowest positive responses are found with the lowest oxygen content. Apparently there is sufficient oxygen here for the usual activities to be carried on at the usual rate and not enough to stimulate them greatly. The length of time the animals have been in the laboratory does not affect their response providing they are kept in conditions resembling as nearly as possible, those in their usual habitat.

The response of these stream isopods to the current is vigorous and usually definite, that is, they are either definitely positive or negative. When the current is reversed they tend to reverse within the first ten seconds after the new current is set up, and often they are all reversed before the stirring is stopped. They also move vigorously, sometimes completing two circumferences of the pan in the minute reaction time allowed. This would mean a rate of about 80 cm. per minute. They sometimes pivot on their posterior end, turning their head in a complete circle before starting a definite reaction and this testing reaction is usually followed by a positive response.

In all these cases the amount of free carbon dioxide in the water was very low. That is, it never exceeded 3 cc. per liter of water and usually ran much lower, the average being about 2 cc. per liter. Thus the variations of the free carbon dioxide are too small to be of any significance, and the fact that it was present in such small amounts will have to be considered in determining the cause of the high percentage of positive reactions.

b. Breeding season

The breeding season of these isopods begins before the ice is out of the water in the spring. It reaches its culmination by the last of April, and gradually diminishes. Occasionally breeding

occurs throughout the summer but is very rare during August and September. As the weather becomes colder, a new period sets in, but this is of much shorter duration than the spring periods. The shortening cannot be wholly due to the increasing coldness because animals brought into the laboratory and kept under normal conditions do not long continue breeding. In these animals in the laboratory, however, a new period of breeding begins about the first of December. Starting with a few individuals it slowly increases in importance until by the middle of January, it is the dominant activity of the animals. Curiously enough this is much more pronounced in animals kept at temperatures about 5°C. than in those at 20°. In these laboratory isopods, the season stops about the time it is reaching its height in the field.

One sign of the approach of the breeding season is the increased tendency to collect in bunches. Bunching is apt to occur at any time during the year if conditions become unfavorable, as when there is a sudden drop in temperature, but the bunching tendency of the breeding season is even stronger. Often these close irregular groupings occur, containing six or eight individuals. This is especially apt to happen when the animals are stirred in a current so that they are thrown against each other. The copulation occurs much as Holmes has described for amphipods (Holmes '03, p. 288). The females may become quite helpless as the brood pouch develops and unless they are clinging to some support, they are often brought to the surface and float around ventral side up, entirely unable to right themselves or to regain the bottom unless they chance upon some solid object.

The effect of the breeding season on the rheotactic response of stream *Aselli* is shown in table 3. The first part of the table gives the cycle of reactions due to the breeding season as shown in the field experiments from April till October. The second part traces the progress of these influences upon laboratory stock during the winter months. One of the most noticeable changes in the rheotactic response is the marked decrease in the percentage of positive responses. Another almost as striking is the extreme variability in animals selected at random from the breeding stock. This variability is not so apparent when the same animals are

TABLE 3

Part 1. Field trials. The effect of the breeding season on the rheolactic response of adult stream *Aselli*

O ₂	RESPONSE			NUMBER OF TRIALS	TEMPER- ATURE (°C)	DATE 1911	REMARKS
cc. per liter	per cent +	per cent -	per cent =				
9.31	38	14	48	50	13	4-6	3 ♀ b.p. 2 ♂ *
7.80	9	74	17	80	9	4-22	4 ♀ 4 ♂
7.55	48	45	7	60	9	4-22	all ♀
7.55	33	60	7	40	10	4-22	all ♀
7.55	40	42	18	50	10	4-22	all ♀
7.23	36	60	4	70	11	1-29	all with b.p.
7.23	12	41	44	50	11	1-29	all ♂ small
7.23	50	32	18	50	11	4-29	all ♂ large
7.28	32	58	10	50	14	5-16	mixed
7.28	48	52	0	50	14	5-16	mixed
1.02	14	80	6	50	18	5-21	mixed; no bp.
1.02	11	63	26	50	18	5-21	5 prs. cop.
5.30	42	52	6	50	18	5-21	4 ♀ 1 ♂
4.50	48	52	0	40	18	5-21	mixed
4.65	0	70	30	20	18	5-21	2 prs. cop.
5.63	60	40	0	10	23	6-8	♀
5.63	68	24	8	50	23	6-8	mixed
5.01	53	42	5	45	16	6-17	3 ♀ 2 ♂
6.26	14	82	4	50	23	7-8	mixed
7.00	88	6	6	50	23	8-1	normal
5.46	78	12	10	50	17	10-4	normal
8.54	50	46	4	50	13	10-5	selected with b.p.

* b.p. stands for females with brood pouches.

Part 2. Breeding season in laboratory stock

6.05	57	40	3	30	18	12-1	copulating
						1912	
7.34	50	47	3	60	4	1-11	1 pr. cop.
9.54	44	20	36	50	6	1-19	mixed
9.54	54	12	34	50	8	1-19	mixed
9.54	53	33	14	50	10	1-19	mixed
9.54	60	20	20	20	8	1-23	mixed
9.54	70	10	20	20	8	1-23	2 prs. cop.
10.47	28	51	21	80	4	1-28	all large ♂
8.73	21	23	56	80	4	1-30	mixed
7.83	30	29	41	80	4	1-13	mixed
8.88	27	30	43	70	6	1-31	mixed
9.11	29	16	55	70	6	2-2	mixed
8.82	27	23	50	70	4	2-1	mixed
8.48	29	23	48	70	6	2-8	mixed
7.97	21	37	42	70	5	2-13	mixed
8.48	32	33	35	60	5	2-17	mixed

tested from time to time. Thus the last trials recorded in part 2 of the table were all made on the same group of animals, with the exception of two cases the oxygen content of the water was high and in all cases the amount of carbon dioxide present as free carbon dioxide was very small, so that these changes in reaction are not due to a modified gas content of the water.

It will be seen in later experiments, that the degree of positiveness depends upon the metabolic rate of the animals. That is, in animals having a high rate of metabolism there is a high positive response. From this point of view the decrease in the positive responses would be accounted for by assuming that the animals are in a state of lowered metabolism during the breeding season. This view is supported by those plants and lower animals that reproduce asexually during conditions favorable for growth and respond to poorer external conditions by sexual reproduction.

The cause of the variation in response is not impossible of solution although at present it cannot be treated entirely from the experimental side. From the results given it is evident that during the breeding season not all isopods are in the same physiological condition at any one time. The results from any one day as listed in the table show wide variations, yet these were taken from almost identical external conditions, so the variable quantity in this case must be an internal one. This view is further supported by comparing those results where the animals were in the copulating position. This term is used in the table to show when the females were being carried by the males. There are three cases given when all the individuals tried were in this position. Of these one gave a positive response of 11 per cent, another of 70 per cent, and the third gave no positive response at all. The first were taken from a very low oxygen content and as will be seen later this tends to decrease the positive response. Yet with this added complication they gave a much higher response than did two pairs taken the same day from another place in the same stream. It may be that in the case where no positive response was given the animals were near the actual copulation time, and the animals giving the 70 per cent response may have been far from this period.

The large brood pouch on the ventral side of the females offers a serious mechanical obstacle to making progress against a strong current, but since the same reaction tendencies occur in the males this mechanical hindrance cannot be the only factor in the response. The condition of the germinal glands during the breeding season and the exact connection existing between their activity and the rheotactic response will be presented in detail in another paper.

In comparison with the normal behavior, the large increase in indifference to the current is remarkable, since this is just the condition that tends to prevail in pond animals. The action in the current is also decidedly different. The animals are much more easily swept from their footing; they do not reverse so rapidly as the current changes, often failing to reverse at all and the speed of reaction is greatly lowered. In some cases there is no response at all; the animals are then in the same state as that caused by the strongest depressing agents.

This breeding behavior brings up some interesting points in the ecology of the isopods. Although they are taken in streams they are rarely found in rapid parts of permanent ones, being limited for the most part to the pools and protected places. Outside of the breeding season they are fitted by their positive reaction to the current and their strong clinging ability to maintain themselves in much stronger currents than those in which they are found. The reason for their absence in these places must be due to the influence of the breeding activities upon their behavior. This is especially significant since their period of least ability to maintain themselves corresponds to the time of the strongest current in the stream. Hence their breeding behavior limits them to those streams where they can find ample lodging places during this time of weakened responses.

c. Juvenile mores

When the isopods are first liberated they are about 2 mm. in length and usually give no response to the current but cling passively to the bottom. Consistent rheotactic responses were made by the time the animals were about 3 mm. long; that is when

they were about a month old. As table 4 shows, the general positive response of the young isopods is much lower than that of the adults but that the positiveness increases with age.

Again there is more variation in the positive response than seems consistent, for, as the table shows, the isopods give at times a low response regardless of size and of the external conditions here controlled.

It will be noted that the increase in positive reaction was not due primarily to the oxygen content of the water because this was high when the positiveness was low. The carbon dioxide was also low as it has been in all cases discussed. Evidently the growth in size was the most important factor in the increased positive response.

TABLE 4
Rheotaxis in juvenile stream Aselli

SIZE	O ₂	RESPONSE			NUMBER OF TRIALS	TEMPERATURE, C.
		per cent +	per cent -	per cent x		
mm.	cc. per liter					
3.5	6.26	33	67	0	40	17
4.0	7.28	8	88	4	50	21
4.0	5.63	16	84	0	25	17
4.0	5.63	40	36	24	50	20
4.0	6.55	42	48	10	30	17
5.0	7.28	14	80	6	50	21
5.0	7.28	30	68	2	50	21
5.0	5.01	40	50	10	50	12
5.0	5.23	45	52	3	40	21
5.0	6.38	55	20	25	20	17
5.0	6.94	60	30	10	50	15
5.5	5.63	19	75	6	80	23
5.5	7.06	50	40	10	60	22
5.5	7.06	55	36	9	80	22
6.0	5.63	10	84	0	90	23
6.0	5.63	15	75	10	60	17
6.0	8.19	32	14	54	50	8
6.0	5.23	45	52	3	40	21
6.0	6.57	90	7	3	40	22
6.5	7.00	88	6	6	50	23
6.5	1.14	92	0	8	50	29
6.5	1.14	60	28	12	50	20
8.0	5.23	66	42	2	50	21
8.0	6.36	75	25	0	40	19
8.0	4.27	90	5	5	20	21

The importance of this factor is emphasized by the two trials where with a low oxygen content the animals tested gave a high positive response. This is the one case in the progress of this work where the laboratory tests have failed to run parallel with the field results, for as will be seen later, keeping young stream Aselli in water having a low oxygen content kept the animals from developing a high positive response, in all the cases tried. These two high results were obtained in the field on July 4, 1911. The stream at this time was reduced to a series of small pools with no running water, and in the case of the higher response, the animals were in a very high temperature, 29°C., which may to some extent account for the difference between the two trials. Reference to this will be made in another part of this paper in connection with experimental data which may tend to clear up the case.

d. Response to straight current

Banta ('10, pp. 467-468) described a trough which he devised for testing the response of isopods to a straight current. His apparatus consisted of a simple straight trough in which the current was equalized by passing through a number of wire screens before it reached the experimental part of the trough. He introduced the isopods to be tested into still water and then turned on the current. After some crawling back and forth the animals collected at the upper end and stayed there from fifteen minutes to two days, afterward reversing their reaction.

In order to test the efficiency of the pan response as an index of the rheotactic activity of the isopods, Banta's experiments were repeated. A different type of trough was devised and is shown in figure 2. This trough has a rounded well 10 cm. in diameter.

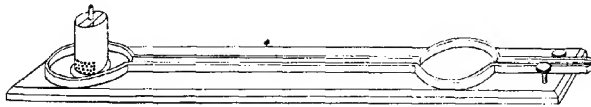


Fig. 2 Straight current apparatus

The water is introduced through a circular tube 3 cm. in diameter, which has holes to permit the exit of the water only on the side away from the trough. The trough is 3 cm. across, 2 cm. deep, and 50 cm. long. At its lower end it opens into another well exactly like the one at the upper end. This in turn opens into a drainway directly below the end of the main trough, the drain being 1 cm. above the general floor of the apparatus. The whole trough is made of wood and is painted a dead black with waterproof paint. Thanks to the careful workmanship of Mr. Floyd of the Ryerson Physical Laboratory, the trough is very accurate in its dimensions. The animals were confined by wire gauze and their movements measured by means of a centimeter scale at the top of one side of the trough. When in use the apparatus was kept almost level.

The animals to be experimented upon, were placed in the current at the center of the trough. They usually started off in the direction in which they were first headed regardless of the response which they would ultimately make. In some cases positive animals would continue with the current to the lower end and then turn and make their way back along the edge of the trough, toward the upper end. Usually they did not collect in contact with the upper screen as Banta found to be the case in his experiments. Rather the response brought a majority to the upper part of the trough where they settled in the angles between the bottom and the sides, with some of course clinging to the screen. In taking readings the exact position of each individual was recorded and an average taken of the position of the whole group. From five to eight was found to be the most convenient number to be tested at one time. A summary of the results obtained in this manner, with normal stream Aselli, is found in table 5.

Often the isopods would move first up stream and then down stream, without giving a definite reversal. In these cases the reversal time was taken to be the time after which there was no decided movement against the current. When the experiments ran over night no readings were taken after midnight. Except in the third and last experiments recorded in the table there was

TABLE 5

Stream Aselli in straight current

O ₂ cc. per liter	TIME BEFORE REVERSAL	RESPONSE per cent +	LENGTH OF EXPERIMENT	CURRENT STRENGTH	TEMPERATURE	
					Trough	Aquarium
9.24	after 2:01	84	3:06	1680	6	7
9.21	2:47	78	3:59	1680	17	7
9.52	after 5:00	54* •	15:52	1000	5	8
	5:30	70	29:28	1020	5	8
9.03	7:40	74	29:37	1050	8	10
5.13	23:48	64	59:48	1050	7	10
6.33	11:45	58	35:15	1020	12	15
	5:17	32*	44:47	1030	14	14
4.33	4:35	60	5:17	1100	16	16
	6:40	77	24:07	1050	16	16
7.00	6:19	78	10:35	1250	18	18
6.85	5:20	78	7:45	1100	17	16
6.77	after 7:00	86	26:20	1050	13	15

* Pan response taken after trough reversal; all others were taken before. The first column gives the oxygen content of the water in cc. per liter. The second gives the time in hours and minutes before there was a decided reversal in the response to the current. The third column shows the percentage of positive responses of the same animals to the circular pan current. The fourth column shows the duration of the experiment in hours and minutes. The strength of the current is given in cubic centimeters of flow per minute, the trough being placed as nearly level as possible. In the three cases where no exact amount of oxygen is shown, the amount present was well above air saturation at the given temperature.

no appreciable change of position during this period when no readings were taken.

A study of table 5 will show that there is a relatively long lapse of time before reversal. This length of time is correlated with the high per cent of positive responses. This correlation is by no means close, nor does it follow the fluctuations of the pan response but the significance of the relation between the two is well shown by comparing the results obtained here with those of isopods kept in water having a low oxygen content.

2. POND ASELLI

a. Adults

As has already been mentioned, there are two species of isopods found in the ponds near Chicago. *Mancasellus danielsii* (Richardson, '05, pp. 417-419) has been previously reported from LaPorte, Indiana, only. It is a much flattened form and is generally found in the grasses in the shallower water of the ponds. Its reactions so far as tested agree with those of *A. communis* and unless otherwise designated all the discussion of pond behavior will be based on the latter species.

The pond Aselli are decidedly smaller than those from the stream. In the isopods that have been measured the difference averaged about 3 mm., that is they were about 75 to 80 per cent of the length of the stream forms. However as has already been stated the pond isopods contain all the variations of the propodus of the first pair of legs that are to be found in the stream forms. The pond isopods react to light, heat, touch, and gravity in much the same way as the stream animals, although the speed of the reaction and the sensitiveness to the stimuli are probably different. The rheotactic response of the isopods from the two habitats is markedly different. In place of the positive reaction to the current dominating as in the stream mores, these isopods give a high proportion of indefinite responses. Their orientation is less definite and they do not appear to be so capable of holding an orientation once it is attained. Their response is less vigorous than that of the stream isopods and they are much more easily swept off their feet by the current.

A typical pond isopod response to current is shown in figure 3. This trial and record was made exactly like that for the stream isopod shown in figure 1. The result of twenty trials is given in which the animal went positive 25 per cent, negative 30 per cent, and indifferent to the current 45 per cent of the total number of trials.

The same response is shown in table 6. It will be noted that the table includes the response made during the breeding season and that this response is not markedly different from that of the

rest of the year although it is somewhat less positive. That is, the depression found in the stream Aselli during the breeding season is not so marked in the pond isopods.

The external condition with which the low positive response seems to be correlated is the low oxygen content of the water.

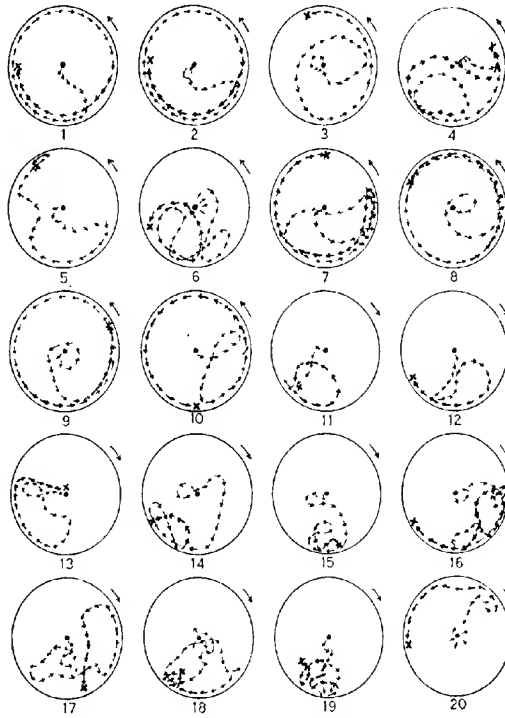


Fig. 3 Twenty successive rheotactic reactions of one pond *Asellus*

Thus the highest oxygen content of the pond is a little below the lowest found in the stream during a period of normal response, while the normal amount of oxygen found in the pond water is much below that of the stream. The amount of free carbon dioxide present in the pond water is higher than in the stream, but

TABLE 6
Rheotactic response of adult pond Aselli

O ₂	RESPONSE			NUMBER OF TRIALS	CO ₂	TEMPER- ATURE	REMARKS
cc. per liter	per cent +	per cent -	per cent x		cc. per liter		
2.17	16	24	60	50	29	3	in lab. 14 hours
2.17	5	23	72	40	29	8	24 hours later
1.57	18	16	66	50	31	12	in lab. 33 hours
5.04	36	34	30	50	14	15	
2.28	17	19	64	80	9	13	field: all males
2.28	19	21	60	80	9	13	field: all females
2.28	8	20	72	25	9	13	field: conjugat- ing
1.08	32	30	38	50		13	all with b.p
0.95	20	14	66	50	26.5	23	4 males: 1 fe- male, b.p.
3.13	44	40	16	50		22	close spring breeding
2.33	20	48	32	40		8	7-25-11: in ice chest
3.66	36	18	16	50		15	10-8-11
4.49	35	50	15	60		6	10-28-11
1.25	32	28	40	50		18	lab. 10-19-11
1.53	38	28	34	50		18	lab. 11-28-11
2.41	25	30	45	775			

different sources of evidence tend to show that this amount of carbon dioxide is not sufficient to account for the difference in behavior. This problem will be considered later under the experiments upon the effect of carbon dioxide.

b. Juvenile pond isopods

The young isopods were tested for their response to current when they were about forty-five days old. At that time they were 3 mm. in length. As was the case in the stream animals they gave a low positive response, behaving in almost every respect like stream isopods of the same size. But as will be seen in table 7 the later response did not show the increase in positiveness that was found in the developing stream forms. The other

facts shown by the table support the experiments already discussed. The two responses marked 'M.d.' in the table show the reactions of juvenile *Mancasellus danielsii*. These trials were made in spring and summer and the two instances of lower temperature, are due to the animals being kept in an ice box. However, they were left in each case long enough to become thoroughly acclimated and so showed no effect of the continued low temperature.

c. Response to straight current

Only five trials have been made with pond isopods from water with a low oxygen content but as was the case in the stream animals these support the pan reactions, in that they show a short period of positive response to the straight current correlated with the low response in the pan reactions. A summary of the reactions is given in table 8.

The conditions of the trials were exactly like those already described for this method. Owing to imperfect apparatus it was not possible to keep the temperature exactly the same in these trials and the variation in the second and fourth tests is enough to cause some difference in the response. In both cases this would tend to shorten the positive reaction if it acted in a

TABLE 7
Rheotaxis in juvenile pond Aselli

SIZE	O ₂	RESPONSE			NUMBER OF TRIALS	TEMPERATURE	AGE IN DAYS
mm.	cc. per liter	percent +	percent -	percent a			
3	2.5	20	39	41	70	19	40 (approx.)
3	0.95	3	7	90	30	23	field test
3	2.33	23	36	41	100	24	56
3	3.13	40	55	5	80	21	75
4	3.24	24	60	16	50	25	122
4	2.23	33	33	34	30	8	116
5	3.24	14	57	29	50	25	122
6	3.71	38	42	20	100	20	193
4	3.13	32	56	12	50	22	M.d.*
7	2.33	20	6	74	80	8	M.d.

* *Mancasellus danielsii*.

TABLE 8
Pond Aselli in straight current

O ₂ cc. per liter	TIME BEFORE REVERSAL *	PAN RESPONSE	LENGTH OF EXPERI- MENT	TEMPERATURE		PREVIOUS CONDITIONS
				Trough	Aquarium	
2.17	†	16	22.92	4	3	in lab. 12 hours
2.17	0:24	5	1.00	4	10	in lab. 40 hours
2.20	2:08	18	19.03	4	3	in lab. 3 hours large males
5.04	1:04	36	2.55	5	15	in lab. 18 hours
5.86	0:41	†	23.36	11	11	in tap water 9 days; b.p.

* Time before reversal and the length of experiment are given in hours and minutes; the pan response is in terms of the per cent of positive reactions. The current was kept at 1040 cc. per minute.

† With brood pouches unable to stay on bottom.

‡ Almost no positive response at all.

direct way. But if there were a shock effect the result would be a lengthened response, so that perhaps the two might offset each other to some extent. Then, too, the experiments were in running water with a large amount of oxygen, because at this time there was no means of controlling the amount of oxygen present in water flowing at the rate used here.

The animals used in the first, second, and last trials were kept in running tap water for the length of time they had been in the laboratory. In the first two cases the animals were not exposed long enough to cause any effect upon their reaction, so the oxygen content of their original habitat is given. In the last trial the high oxygen evidently had not made any change in the response of the isopods, but this may have been due to the fact that these were females with large brood pouches that made it impossible for them to keep their footing in the strong current.

3. SUMMARY OF RHEOTACTIC RESPONSE GIVEN UNDER NATURAL ENVIRONMENTAL CONDITIONS

In discussing the normal rheotactic responses of *A. communis* it has been shown that the same species is found in both ponds and streams, but that it is limited in the latter to those having

quiet pools or other places of lodgement which keep the isopods from being swept down stream in the breeding season. The normal rheotactic reactions of animals from the two types of habitats are quite different. Animals from streams give a high percentage of positive responses; they are much more vigorous and definite in their reactions and show a long period of positive reaction in a straight current. The pond isopods on the other hand give a weak positive response and are much inclined to be indifferent to the current. They are less active than the stream mores, and in the straight current, they give only a short positive response.

The most obvious difference in the environment in the two habitats is the difference in the oxygen content of the water. In the streams this is normally between 5 and 10 cc. per liter depending on temperature, rate of flow, and the character of the stream bed. In the ponds studied, on the other hand, the amount of oxygen present is low, seldom going above 3 cc. per liter at any period of the year. There is also a difference in the amount of free carbon dioxide present in the two habitats. This runs about 2 cc. per liter in the streams while in the ponds it has been taken as high as 40 cc. per liter, the usual amount however is about 10 to 15 cc. per liter. From this work alone it would seem probable, that the difference in positive response to the current in the two habitats may be due to the difference in the oxygen or carbon dioxide content of the water.

The main breeding season occurs in the spring of the year in both habitats. In the pond isopods, this does not affect the normal rheotactic response so markedly as with the Aselli from the stream. In these it causes a distinct lessening of the positive reactions so that for the time being, they behave as though they were pond isopods. The young from both habitats give a low positive response, but in the streams the reaction becomes more positive as the animals increase in size, while in the pond isopods it remains the same throughout life.

III. RHEOTACTIC RESPONSE UNDER EXPERIMENTAL CONDITIONS

1. STREAM ASELLI

a. With decreased oxygen

The methods of handling the problem of confining the isopods in water having a low oxygen content, have varied more in the progress of this work than those in any other line. The first method tried was to keep the animals in an aquarium having no inflow of water and containing a large amount of dead leaves and other organic matter, which would absorb oxygen. This worked after a scum had appeared to hinder the absorption of oxygen from the air. Obviously, this method at best was crude, and after trying boiling and cooling water in ordinary vessels, which was of course very laborious, a machine for deaerating water was devised. This apparatus was devised and built by V. E. Shelford and the writer and will be described in detail elsewhere. In brief, it consists of a tower down which the water runs through successive sieves and is thus reduced to air saturation at the temperature used. Then the water is heated in aluminum pans over powerful gas flames until it is about to the boiling point. It is then cooled by passing through coils of block tin pipe surrounded by tap water. In this way 1200 cc. of water can be treated each minute and the oxygen content reduced from 8 cc. per liter of water to less than half a cubic centimeter. By arranging the flow in the cooler any desired temperature can be obtained. A gas introducer permits the addition of oxygen or carbon dioxide to the deaerated water.

Since the completion of this device the best results with low oxygen have been obtained and most of the data to be given on this subject are from work done with water prepared in this way. The most important chemical changes due to the boiling, as shown in analyses by Mariner and Hoskins of Chicago, are seen in table 9.²

In this work the animals to be used were first tested and then placed in glass jars with ground glass edges. A glass plate was

² The complete table will be published later.

TABLE 9

The effect of the deaerating apparatus upon the salt content of the water. A = Boiled in apparatus. B = Hot tap water. C = Cold tap water. Results are given in parts per 100,000

	A	B*	C
Nitrates.....	0.060	0.170	0.070
Iron.....	0.011	0.015	0.006
Lime (CaO).....	4.360	4.360	4.840
Magnesia (MgO).....	2.001	1.657	1.882
Sulphuric acid (SO ₄).....	0.0071	0.003	0.004
Total solids.....	14.800	13.800	15.400

In the experiments the hot water (B) from the University heating system was treated in the apparatus and the table shows that this treatment made it more like the ordinary tap water, as far as the contained salts are concerned.

used as a cover and was sealed down with vaseline, care being taken to avoid enclosing any air bubbles. In experiments that were run some time a few leaves were placed in the jar for food. The water was changed often enough to provide an even supply of oxygen.

(1) *Normal adults.* Table 10 shows the results of a number of trials in which, with the exception of no. 9, the oxygen content of the water was low at the time of the trial and had been low for at least ten days. The purpose of the table is simply to show the typical response of stream isopods that have been kept in low oxygen. It will be noted at once that the reaction is decidedly different from that of the normal stream Aselli, in that the positive percentage is lowered and the amount of indifference to the current is correspondingly increased. It is significant that the lowest positive responses are made by the animals in the lowest oxygen, and that above 3 cc. the response becomes more variable although in the main it increases as the oxygen supply increases. The reactions that are grouped under no. 9 in table 10, were given by isopods that had been in running water up until twelve days before this trial was made. At that time they were giving a 70 per cent positive response in an oxygen content of 5.92 cc. per liter. The water was turned off and three different trials made, with the results recorded. From the variation, this seems to be

TABLE 10

The effect of low oxygen upon the rheotactic response of adult stream Aselli

O ₂ cc. per liter	RESPONSE			NUMBER OF TRIALS	TEMPERATURE
	per cent +	per cent -	per cent ±		
(1) 0.23	23	35	42	100	18
(2) 0.37	20	6	74	50	8
(3) 1.00	30	20	50	40	7
(4) 1.90	20	44	36	50	17
(5) 3.37	62	6	32	50	19
(6) 3.50	46	44	10	50	17
(7) 3.58	38	35	27	100	15
(8) 4.16	62	18	20	50	19
(9) 4.44	56	40	4	50	9
	60	30	10	50	
	74	26	0	50	
(10) 4.50	47	28	25	60	19
(11) 4.83	40	50	10	30	22
(12) 4.83	53	40	7	30	22
3.06	45	30	25	760	

the breaking point in the reaction to current for these animals at this time. However if they had been accustomed to a lower amount of oxygen, they might have reacted positively when kept in this amount of oxygen. The effect of increasing the amount of oxygen is shown by the fact that after one day in 7.51 cc. oxygen per liter, these isopods gave an 82 per cent positive reaction.

Something of the details of the effect of a lowered amount of oxygen is shown in table 11. The first set of results is taken from the early work. The amounts of oxygen did not rise, during the time shown in the table, above 4.5 cc. per liter nor fall below 2 cc. The effect of the lack of oxygen in this case became evident after the animals had been in quiet water for twenty-four days. An entry in the laboratory notes for that day reads as follows: "The reaction to current is slower, less definite, and is maintained for a shorter period than with the freshly collected isopods." Unfortunately the exact oxygen content at that time is unknown. The most important thing shown by this section of the table is that the *Aselli* tend to become acclimated to the low oxygen and at length react with a normal amount of positiveness.

TABLE 11
The effect of continuous action of low oxygen upon rheotaxis in adult stream Asellus
I. In open aquarium at room temperature (18°-21°C.) stock started 10-12-10

EXPERIMENT				CONTROL			
O ₂	RESPONSE	NUMBER OF TRIALS	TIME EXPOSED	O ₂	RESPONSE	NUMBER OF TRIALS	
cc. per liter	per cent —	per cent +	days	cc. per liter	per cent —	per cent +	
3.37	62	32	53				
4.16	62	18	76				
3.98	62	0	89				
2.27	97	0	210				
No special control. For general control, see table 2							
II. In sealed glass jars, started 10-23-11; kept at room temperature							
3.50	46	44	field				
4.50	47	28	field				
0.23	23	25	9 days				
6.84	52	36	53 days				
III. In sealed jars, 7°-9°C.; started 11-2-11							
2.56	38	32	9	5.92	70	24	
2.12	80	12	20	7.54	73	27	
0.37	20	6	45				
1.00	30	20	46	9.37	74	16	
IV. In sealed jars, at room temperature; started 1-30-12							
8.14	85	11	4	8.14	79	21	
0.98	53	40	7	6.15	76	14	
0.63	43	33	24	6.77	86	13	
0.11			7	7.00	70	22	
			all dead			8	

with an amount of oxygen that at first caused a decided lessening of the positive reaction.³

The second part of the table shows an interesting case of the correlation between the amount of oxygen present and the positive response of the isopods. The first two field trials show a medium amount of oxygen present and a correspondingly medium positive response. The third trial shows the effects of nine days in the laboratory in which time the oxygen had been almost all used and the positive response is halved as a result. Then with an increase in the amount of oxygen, the positive reaction increased. In both of these cases the control was furnished by keeping the same animals in running water, and as was seen in table 2, those isopods stayed positive throughout as far as oxygen was concerned.

In the next two parts of the table, however, the control was kept under exactly the same conditions as the experiment, except as regards the oxygen content. Part 3 shows both the acclimatization effect and the dependence of the positive reaction upon the amount of oxygen present. Part 4 gives some idea as to the rate with which the effect of a lowered amount of oxygen may be reflected in the rheotactic response.

The amount of carbon dioxide free in the water was never high in these experiments. The highest record is that of 4.5 cc. per liter which occurred in the experiment given in part 1 of the table. In the last experiment given, the carbon dioxide did not rise above 1 cc. per liter in the experiment while in the control it was between 1 and 3 cc. to the liter. Neither can the boiled water have been a factor in these changes, for the isopods used in the last case, both control and experiment, had been kept for a month in the boiled water that had been saturated with oxygen by absorption from the air. For that matter, the reactions given in table 1 were given by isopods from this re-aerated boiled water, and it will be remembered that their reactions averaged 89.4 per cent positive.

³ Haldane and Smith ('97, p. 250) report a similar acclimatization occurring in mice when subjected to a decreased oxygen tension.

From these results it will be seen that a decrease in the oxygen supply decreases the positive response of the isopods to current and that this decrease may be only temporary, or in other words that the Aselli may become acclimated to the new conditions and give a high positive response in an oxygen content that at first caused a marked decrease in their normal reactions. However, at no time in the progress of these experiments, have the isopods showed any sign of an acclimatization when kept in an oxygen content of about 1 cc. per liter. Thus the stream isopod if kept continuously in the average amount of oxygen to be found in the ponds would in time come to respond positively to currents under the new conditions, yet, if they were subjected to the extremes of low oxygen that are sometimes maintained for fairly long periods of time, there is no evidence that adults would ever become acclimated.

These results are of importance when considered in the light of the conditions which the stream isopods may have to meet in nature. As has already been said, they live in pools of small streams. In summer these streams sometimes cease to run and in this condition, the pools often take on the characteristics of a pond for the time that the condition lasts. The cycle of reactions under these conditions is easily seen. The isopods used to a super-saturated oxygen supply would become less positive to a chance current as the pool water became low in oxygen. Then unless the deoxygenation went too far, there would be an acclimatization so that on the average when the stream began flowing again the isopods would be positive to the current and would thus be better equipped to escape being carried out of the stream. Of course even when they are negative to the current, many would be kept in the stream by being stranded against piles of drift.

(2) *Breeding season.* But one controlled experiment was run on the effect of lowered oxygen supply on the breeding season response, but this appears significant in that the experimental results confirmed the prediction that was made concerning them. They also ran parallel with the effect of an increase in the amount of carbon dioxide as will be shown in a later section. The animals used in this experiment were large males about 15 mm. in

length. All were as near the same size and general condition as could be selected from their external appearance. The stock had first shown signs of the approach of the breeding season in the latter part of December and these trials were begun January 31, 1912, so that at that time they were in the midst of the breeding season.

The isopods were taken from a temperature of 4° C. and with the exception of the first trial in the control they were kept within two degrees of this temperature throughout the experiment. The heightened response obtained from the control in their first trial may be due to the fact that the temperature was 4° above that to which they were accustomed, or it may mean that the isopods used were not so completely under the influence of the breeding response as they became later. It will be noted that during the time the experimental animals were giving a changed reaction, the control gave almost no variation in response. •

In the experiment the variation in the first two days is not strong enough to make one sure of the cause without more data, but at the end of the third day there is a strong increase in the positive reaction which is entirely different from anything that we have yet seen in the response to a sudden lowering of the oxygen content of the water. This increase in positiveness was maintained three days in which time it had increased until the response was almost that of the normal adult isopod under high oxygen conditions. Then the response fell off and at the end of the experiment it was decreased almost to the zero mark. This decrease started at the time of the highest oxygen supply so that it was not due to the variation of the amount of oxygen present.

The following explanation of these phenomena is suggested. When the breeding activity set in, energy normally spent in general body metabolism is taken by the reproductive organs. As these increase in activity, the amount of energy left for bodily activities decreases and hence the positiveness of the animals to currents of water is diminished. But the presence of an external medium tending to decrease the rate of metabolism decreases that of the reproductive system and so gives an increased amount of energy for bodily activities. This results in an increase of the

TABLE 12
Effect of increased oxygen supply upon the rheotactic response during breeding season, of stream Asclli

EXPERIMENT						CONTROL					
O ₂	RESPONSE			NUMBER OF TRIALS	TIME EXPOSED days	O ₂ cc. per liter	RESPONSE			NUMBER OF TRIALS	
	per cent +	per cent -	per cent α				per cent +	per cent -	per cent α		
10.47	28	51	21	80	0	10.47	46	40	14	80	
1.85	36	60	4	80	1	8.73	21	23	56	70	
1.99	31	66	13	80	2	8.88	27	30	43	70	
1.15	50	31	19	80	3	9.11	29	16	55	70	
1.91	51	20	29	80	5	8.82	27	23	50	70	
1.53	64	24	12	80	7	8.48	29	43	28	70	
2.55	40	34	26	80	9	8.68	21	30	49	70	
0.63	37	50	13	70	11	7.97	21	37	42	70	
0.62	19	17	64	70	13	8.48	32	33	35	70	
0.85	6	2	92	50	17	7.17	59	10	40	60	

positive response. Later if the external depressing factors are continued in their action, the rate of metabolism is cut below that which supplied energy for the body alone and this results in a decrease in positiveness below that usual in the breeding season. Besides being supported by the rheotactic response this hypothesis is supported by the fact that the isopods tend to lose their general breeding reactions if kept in a decreased oxygen supply. The hypothesis is capable of being experimentally investigated, and this is one of the points upon which the writer expects to do further work.

(3) *Juvenile stream Aselli*. It has been shown in dealing with the rheotactic response of juvenile stream isopods in the field (table 4) that in at least one case the Aselli are known to have given an increase to a strong degree of positiveness in the presence of a small amount of oxygen. All the tests that have been carried on in the laboratory have failed to give a corresponding result. These experiments are of two kinds. First, the less carefully controlled type of experiments that characterized the early part of the work. In these experiments the actual amount of oxygen present in the water was not under control although it was always below air saturation at that temperature. Results from this type of experiment are shown in the first part of table 13. The other experiments were more carefully controlled and these results are shown in the second part of the same table.

In the first part of table 13 the results given for the response of the isopods under high oxygen conditions have been summarized from table 4. That is, the average response shown in that table has been taken as giving the reaction of the isopods of that size under normal conditions. The oxygen content given is also the averaged amount shown for the same trials in the same table. It will be seen that until the isopods are about 5 mm. long, they tend to give the same response regardless of the amount of oxygen supplied, but that after that size is reached, the animals with the higher oxygen supply increase much more rapidly than do the ones with a sub-normal supply. The figures in this case for the animals in the low oxygen content represent the entire life history of the isopods up to the time of their first breeding season.

TABLE 13
The effect of low oxygen upon rheotaxis in juvenile stream Aselli. Fifty trials in each case; temperature 17°-23°C.
Part 1

OXYGEN BELOW SATURATION				OXYGEN ABOVE SATURATION			
SIZE	O ₂	RESPONSE	AGE IN DAYS	SIZE	O ₂	AV. POSITIVE RESPONSE	AGE IN DAYS
mm.	cc. per liter	per cent +	per cent -	mm.	cc. per liter		
3	4.33	34	64	3.5	6.26	33	
3.5	0.57	24	68	4	6.28	22	
4	5.01	50	45	4	6.23	37	
5	3.99	36	6				
5	3.99	29	22	6	6.25	35	
6	4.00	28	0	8	5.75	73	
8	4.90	20	44	8.5	7.80	89.4	
8.5	2.27	60	23				
				Part 2			
3.5	3.64	39	45	4	5.68	18	49
4	3.21	22	61	5	5.23	45	106
4	3.71	24	70	6	6.57	39	159

That is, in the isopods in low oxygen content all their lives there was less tendency to regulate their response to that normal for stream isopods than was the case with their parents that were kept in the same vessel with the second generation. Another point is significant in this connection. The isopods in the smaller amount of oxygen were seven months old at the close of the trials shown in the table, while those in higher oxygen were only five months old. The exact meaning of this time difference is masked by the fact that the isopods in the low oxygen content had been kept in quiet water which was only infrequently changed, while the others were from running water. The food conditions in the two cases were about the same.

The second part of the table summarizes the results of more exact tests. The isopods used in both this experiment and the control were reared in the laboratory from the same stock. They were separated when too small to be tested and those that were to be kept in higher oxygen were placed in an aerating device patterned after that of Colton ('08, p. 428). In the form used this consisted of a large wide mouthed glass bottle which could be tightly sealed (fig. 4).

Two glass tubes extend through the cork. One of these (*B*) opens above the water in the bottle and is connected at the other end with a filter pump. The other glass tube (*A*) is drawn to a fine point at the outside end and only a small opening is left for the entrance of air. In this way the bubbles are smaller and so create less current in the water. On the inside this tube extends down well towards the bottom and opens into a larger tube (*C*). The large tube is about two centimeters in diameter and slopes upward until its upper end extends out of the water. Just below the water's surface are three openings (*E*) blown into the wall of the tube. When the suction is turned on, the air is drawn into the bottle through (*A*). The bubbles are then drawn into (*C*) at (*D*) and escape at (*F*).

During the passage through the water some of the gases of the air are absorbed and in this way the water is kept at air saturation. Whatever current is set up by the passage of the bubbles escapes through the holes (*E*) on the under side of the large

tube. In this way it is possible to keep water well aerated without setting up strong currents. In fact by adjusting the rate of flow of the bubbles and by keeping the tube from extending too far into the water it was found that scarcely any current was noticeable on the bottom of the container.

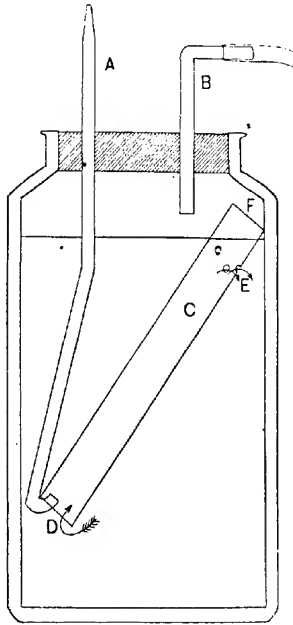


Fig. 4 Modified Colton aerating apparatus

This device has several advantages as a control in this kind of experiments, for it enables one to keep the isopods in a well-aerated, current-free medium. This avoids the possible mechanical stimulation of the current and at the same time keeps the isopods in the presence of their own waste products and thus in the same conditions as prevail in the experiment, except as regards the oxygen supply, and the possible increased oxidation of the waste.

In the case of these experiments, the isopods were put into this aerating device in the water in which they had been reared and this water was not changed. From table 13 it will be seen that the isopods in the aerated water, not only responded to currents with a much stronger degree of positiveness, but that their rate of growth was more rapid. In this case the supply of oxygen is the only factor known to have varied.

(4) *Response to straight current.* •The effect of keeping stream isopods in low oxygen is more marked if tested in a straight current, than when only the pan responses are compared. The lack of adequate apparatus in the early part of the work is more clearly shown in these experiments than when the pond reactions were being discussed. All of the trials except the second and fifth shown in table 14, were made before the later apparatus was built. In these cases it was impossible to control the oxygen supply or the temperature during the trough tests. It was therefore necessary to test the isopods from low oxygen and room temperature, in cold tap water having a high oxygen content. Under these conditions it is remarkable that the results in the rest of the table compare as well as they do with the results obtained under properly controlled conditions.

TABLE 14
Stream Aselli from low oxygen in straight current

O ₂ cc. per liter	TIME* BEFORE REVERSAL	PAN RESPONSE	LENGTH OF EXPERIMENT	CURRENT STRENGTH	TEMPERATURE	
					Trough	Aquarium
(1) 3.96	0:23	18	3:47	1680	17	18
(2) 3.57	0:28	28	26:57	1050	11	11
(3) 3.80	0:33	62	1:02	1000	16	18
(4) 1.00	0:57	28	2:14	1020	15	17
(5) 0.57	1:00	43	3:00	1050	13	15
(6)	1:03	20	4:19	1000	18	13
(7) 3.96	1:12	62	2:28	1680	5	18
(8)	1:22	36	2:43	1000	5	19
(9) 2.28	1:52	20	18:35	1050	10	17
(10) 2.28	6:23	78	18:35	1050	10	17

* As before, the time given is in hours and minutes. The pan response is stated in terms of the per cent of positiveness. The strength of the current is given in cubic centimeters of flow per minute. In the sixth and eighth trials the amount of oxygen was well below the point of air saturation.

In the third, fourth, seventh and eighth trials listed the response was probably shortened by the change in temperature. In all but the second and fifth trials it may have been lengthened by the presence of a large amount of oxygen in the water. In spite of these difficulties the reactions are fairly consistent with the other straight current tests, that is there is an apparent correlation between the length of the positive response and the per cent of positive reactions in the pan tests. This means that the animals in the stream if caught in a sudden current, after a period of low oxygen in summer pools, would maintain themselves against the current for only a short time and must be washed down stream much sooner than isopods from the usual stream conditions.

If the low oxygen should be long continued, however, the animals would tend to become acclimated. This has already been shown to be true for the circular current and is illustrated here by the response given in the tenth trial shown in table 14. This response was given by the same isopods that showed acclimatization to the circular current in the first part of table 11.

b. Carbon dioxide

Not enough experimental work has been done on the effect of increasing the amount of carbon dioxide present in the water to enable this discussion to be critical. However, there are some lines of evidence that appear significant. In the streams the amount of carbon dioxide present in the free state is low, that is it averages from 2 to 3 cc. per liter. In the ponds it has been taken as high as 40 cc. per liter but the average is about one-fourth this amount. Since water will absorb over 900 cc. of carbon dioxide per liter, it will be seen that only a relatively small amount is present even in the ponds.

Part 1 in table 15 gives the effect of low oxygen and increased carbon dioxide, the latter being present in the amount that was found in nature in the most extreme cases. The positive rheotactic response gradually fell under these conditions, but by referring to table 11, part 4, it will be seen that the decrease is not so rapid as when only low oxygen was acting. The second part of the table gives the effect of a much larger amount of car-

TABLE 15
The effect of high carbon dioxide upon rheotaxis in adult stream Aselli
Part 1. Oxygen lowered and carbon dioxide increased. Temperature 15°-18°C.

EXPERIMENT					CONTROL					
O ₂ cc. per liter	CO ₂ cc. per liter	RESPONSE		NUMBER OF TRIALS	TIME EXPOSED	O ₂ cc. per liter	CO ₂ cc. per liter	RESPONSE		NUMBER OF TRIALS
		per cent +	per cent -					per cent +	per cent -	
8.14	2	83	12	5	0	8.14	2.0	79	21	0
1.91	38	89	7	4	2 days	6.15	3.0	76	14	10
0.68	45	65	25	10	4 days	6.77	1.0*	86	13	1
0.11	25	33	23	44	7 days	7.00	2.0	70	22	8
Part 2. Oxygen normal but carbon dioxide very high. Temperature 4°-6°C.										
7.11	227.5	28	12	60	12 hours	10.25	3.0	78	18	4
3.87*	262.0	18	3	79	21 hours	9.05	4.5	67	30	13
6.67	212.5	40	5	55	8 days	7.37	7.0	50	47	3

* The decrease in oxygen is not sufficient to account for the cut in the positive response, in this length of time.

bon dioxide than has been found in any isopod habitat so far studied. In this case the action of the carbon dioxide in twelve hours cut the positive reaction to 28 per cent, while the control in low carbon dioxide stayed at 78 per cent. The control and experimental animals were then changed. In twenty-one hours the isopods in the experiment gave an 18 per cent positive response while those that had been changed from the experiment to the control had increased from 28 to 57 per cent positive. The lowering of the oxygen in this case is due to bubbling the carbon dioxide through the water in order to increase the amount of carbon dioxide present.

Eight days later the response in the two conditions was about the same but the breeding season had set in and one pair of the control animals was copulating. This complication makes the cause of the increased response in the experiment problematical. It may be due to acclimatization, or to an interaction of the carbon dioxide and the breeding season, similar to that given with decreased oxygen.

Part 1 of table 16 shows two experiments run during the breeding season of 1911. The first was started April 6, and the second twenty-four days later. The presence of the carbon dioxide caused an increase in the positive rheotactic response from 38 to 58 per cent in that time. In the second experiment the control animals increased their positiveness due to the close of the breeding activities, and the animals in the increased amount of carbon dioxide did the same, although there was twice the amount of carbon dioxide present that was ever found in the ponds studied.

The second part of table 16 gives the results of experiments run at the same time as those given for low oxygen in table 12. In this case there was first a slight increase followed by a decrease in the positive rheotactic response, and as the amount of carbon dioxide was increased the positive reaction decreased. The increase in positiveness however was not so great as that given with low oxygen under similar conditions.

The results obtained correspond exactly with the known effects of carbon dioxide as a drug. Cushny ('10, p. 588) says in part, that in mammals, carbon dioxide unmixed with oxygen produces

TABLE 16
*The effect of high carbon dioxide upon *Phaenicia* during the breeding season of stream Asell*
 Part 1

EXPERIMENT						CONTROL					
O ₂	CO ₂	RESPONSE	NUMBER OF TRIALS	TEMP. C.	TIME EXPOSED	O ₂	CO ₂	RESPONSE	NUMBER OF TRIALS	TEMP. C.	
cc. per liter	cc. per liter	percent	percent	°	days	cc. per liter	cc. per liter	percent	percent	°	
7.59	45	64	31	5	0	9.31	2	38	14	48	
6.33	37	58	27	15	11						
					24	6.95	1.5	32	58	10	
2.76	82.5	73	15	12	0	5.63	3.0	68	24	8	
					27						

Part 2. Temperature 3°-5°C.

10.47	2.0	38	8	54	80	0	8.82	2.0	27	23	50	70
8.23	50.5	50	35	15	80	2	8.48	2.0	29	43	28	70
8.37	46.5	39	41	20	80	4	8.08	1.7	21	30	49	70
7.83	85.0	30	29	41	80	9	7.97	2.7	21	37	42	70
7.23	105.0	36	33	31	70	13	8.48	0.5	32	33	35	60
5.69	166.0	16	6	78	70	20	7.17	2.0	50	10	40	60
5.75	180.0	0	0	100	50	27	7.23	2.0	13	39	48	60

asphyxia, partially due to a lack of oxygen and partially to a direct depressing action upon the central nervous system. When mixed with sufficient oxygen the specific effects of the gas may be observed without asphyxia. Under these conditions transient stimulation occurs, followed by subsequent depression of the central nervous system and heart. In well diluted vapor, only the exaltation occurs as the anesthesia does not follow. This would mean that the results shown in the first parts of tables 15 and 16 are due to the action of carbon dioxide as a stimulant, and this may even retard the depressing effect of a decreased oxygen supply (table 11, part 4). But when over 200 cc. of carbon dioxide per liter are present, it acts as a strong depressant. Regarding the effect of large quantities, Cushny (l.c., p. 588) says that in mammals a large amount of carbon dioxide probably acts as a poison to protoplasm, for it lessens the amount of oxygen absorbed, so that in the final analysis it would seem that the depressing effect of carbon dioxide is directly due to increased oxidations and thus it acts in the same way as when the supply of oxygen is decreased.

It will be remembered that the free carbon dioxide present in the streams is about 2 cc. per liter, while that of the ponds may run as high as 40 cc. But since in the experiments 45 cc. of carbon dioxide acts as a stimulant, and since small amounts of the gas are known to be mammalian stimulants, it is improbable that the low positive response of pond isopods is correlated with the increased carbon dioxide content of the water. This then leaves the decreased oxygen supply as the main environmental factor with which the lowered positiveness seems correlated.

c. *Chloretone*

Chloretone (acetone chloroform) belongs to the class of substances commonly known as anaesthetics. Authorities generally agree that these anaesthetics inhibit to some extent certain of the fundamental metabolic reactions (Child, '10, p. 173). Different strengths of chloretone were used in these experiments but 0.005 per cent was found to work best for experiments that were to run some time. Preliminary tests showed that the animals collect

in bunches when first placed in a solution containing chloretone. Later they became acclimated and moved more normally but would bunch again if more chloretone was added. As has been previously stated, this reaction is also induced by a sudden increase or decrease in temperature.

The exposure to chloretone for the rheotactic experiments was made in sealed glass jars, the solution being changed every twenty-four hours. The results of one experiment only are shown (table 17) but these are entirely comparable to the reactions given by a number of other tests. In this experiment the control animals remained strongly positive while the reaction of those under the influence of chloretone was cut from 78 to 25 per cent positive. Towards the end of the experiment the animals were evidently becoming acclimated and hence gave a normal response.

d. Potassium cyanide

Potassium cyanide is known to decrease the amount of oxidations by decreasing the ability of the tissues to take up oxygen. (Geppert, '99, p. 208). Then weak solutions of this chemical should show the same results upon isopod reactions as keeping them in a low oxygen supply. Experiments prove this assumption to be true. The results of two series of such experiments are shown in table 18. These experiments were conducted in every respect like those with chloretone. The amount of potassium cyanide used gave only a faint odor to the water.

In the first part of the table, isopods giving a positive path response of 82 per cent, were placed in N/100,000 KCN solution. At the end of five days they gave a 30 per cent positive response. For the next three days they were kept in N/125,000 solution and during that time the response was practically the same. At the end of eight days half of the original number were dead. The remainder were put in tap water and showed a rapid recovery of their normal response. The other trial shows the same results except that when the experiment ended, all but two of the isopods were dead. The lowering of the positive response in the control is due to the fact that the control in these experiments was run with very little food.

TABLE 17
The effect of chlordane upon rheotaxis in adult stream Aselli. 1/200 per cent solution of chlordane used. Temperature 17°-18°C.

O ₂ cc. per liter ^a	EXPERIMENT		NUMBER OF THIALS	TIME IN EXPERIMENT days	CONTROL		NUMBER OF THIALS
	per cent +	per cent -			O ₂ cc. per liter	RESPONSE per cent + per cent -	
5.23	32	25	43	0	5.69	78 16	50
5.23	25	28	47	14			
5.23	25	28	47	14	5.92	70 24	80
4.89	27	51	22	20	5.12	72 20	60
7.00	50	6	44	27			
7.00	42	6	52	27	6.49	89 8	60

TABLE 18
The effect of potassium cyanide upon rheotaxis in adult pond Aselli. Temperature 17°-22°C. Room temperature

KCN	EXPERIMENT		NUMBER OF THIALS	TIME EXPENDED days	O ₂ cc. per liter	CONTROL		NUMBER OF THIALS		
	O ₂ per cent +	RESPONSE per cent -				RESPONSE per cent + per cent -				
N ₁₀₀₀₀₀	3.35	30	40	30	0	7.51	82	17	1	80
N ₁₀₀₀₀₀	6.37	33	37	30	5	5.97	70	18	12	60
N ₁₀₀₀₀₀	7.87	33	40	7	8	6.26	80	14	6	50
Tap water	7.54	73	27	0	30					
Tap water	7.54	27	0	30	14					
Tap water	6.03	80	20	0	30					
N ₁₀₀₀₀₀	5.80	58	16	26	0	7.02	72	8	20	50
N ₁₀₀₀₀₀	6.03	38	0	62	5	6.93	88	6	6	60
N ₁₀₀₀₀₀	5.29	16	36	48	10	5.84	63	2	35	40
N ₁₀₀₀₀₀					15	5.52	58	38	4	50

e. Low temperature

The general effect of lowering the temperature is to cause a decrease in the positiveness of the animals. This decrease is well shown in the experiments listed in table 19. No attempt was made to find how small a decrease or how short an exposure would cause a reaction, the only care being to find if low temperature would affect the response.

The results show that a decrease in temperature does affect the rheotactic response in a marked manner, mainly in that it renders the isopods extremely inactive. Although these experiments do not show any acclimatization, yet by comparing with results listed in table 2 it will be evident that the isopods do come to give their normal positive response at as low as 4°C. above zero. Again this is a reaction to a change of conditions. General experimental work has shown that a change in 3° of temperature does not usually affect the reaction to current, although in one case a change of 4° did have a marked effect. In

TABLE 19
The effect of low temperature upon rheotaxis in adult stream Aselli

TEMPERATURE C.	O ₂	RESPONSE			NUMBER OF TRIALS	TIME EXPOSED TO TEMPERATURE GIVEN
		cc. per liter	per cent +	per cent -	per cent x	
18	5.69	78	16	6	50	7 days
5	8.41	16	84	0	50	field test
18	4.99	94	4	2	50	2 days
4	6.08	42	16	42	50	12 hours
10	7.57	82	17	1	80	10 days
II						
15	7.40	90	8	2	80	over 10 days
0		1	4	95	80	3½ hours
16	6.45	70	14	16	80	4½ hours
III						
12	6.55	64	23	13	80	over 10 days
0		6	5	89	70	2 hours
12	6.50	68	20	14	70	5 hours

a temperature gradient, isopods collect at a temperature near that to which they have been previously exposed so that this optimum shifts with external conditions.

The effect of a decrease in temperature upon the bunching reaction has already been mentioned. Since the isopods become acclimated in both these cases, it was to be expected that they would show a similar reaction regarding the effect of temperature upon their rheotactic response.

f. Starvation

The effect of starvation is shown by the results tabulated in table 20. These experiments were carried on in filtered lake water. The only difference between the experiment and the control was that the latter contained a few leaves for food. The results show that as starvation progressed the positive responses of the isopods were diminished. The decrease shown in the control is due to the approach of the breeding season. As has been explained in other cases this may have helped cause the decrease in the experiment, but by comparison it will be apparent that the breeding season was not the major cause of the change in response.

2. POND ASELLI

a. With increased oxygen

(1) *Normal adults.* It is obvious that if the amount of oxygen present in the habitat is the determining factor in the rheotactic response of isopods, increasing the amount of oxygen present should increase the number of positive reactions. The amount of oxygen present was experimentally increased in three different ways: (1) The animals were placed in running tap water. This method is objectionable because it introduces a current, and the mechanical effect of this might be the stimulating agent; (2) the aerating device already described was used; and (3) the isopods were placed in water containing a large amount of green water moss. Similar results were obtained from all three methods and these results are listed in table 21.

TABLE 26
The effect of starvation upon rheotaxis in adult stream Asellus. Temperature 15°-19°C.

EXPERIMENT					CONTROL				
O ₂	TIME WITHOUT FOOD	RESPONSE			RESPONSE			NUMBER OF TRIALS	per cent α
cc. per liter	days	per cent +	per cent -	per cent α	cc. per liter	per cent +	per cent -		
7.02	0	72	8	20	5.52	70	12	18	50
5.52	15	53	38	4					
<i>I</i>									
7.40	0	90	8	2	6.49	90	10	0	80
6.51	7	41	14	45	8.14	79	21	0	80
6.15	9	20	3	77	6.15	76	14	10	80
<i>II</i>									
6.55	0	75	15	10	6.55	60	33	7	80
5.86	4	52	38	10	4.38	53	43	4	70
6.43	10	53	30	17	5.20	65	22	13	60

TABLE 21

The effect of high oxygen upon rheotaxis in adult pond Aselli

O ₂ cc. per liter	RESPONSE			NUMBER OF TRIALS	TEMPERATURE C.	TIME IN HIGH O ₂ days
	per cent +	per cent -	per cent a			
5.12*	34	24	42	90	17	10
5.12	40	31	29	35	17	11
5.12	32	16	52	50	14.5	19
5.12	46	10	44	50	14.5	19
8.75	43	27	30	30	13	14
9.31	20	28	52	50	11	54
8.96	56	4	40	50	7.5	75
8.96	44	6	50	40	7.5	73
9.22	52	2	46	50	8.5	89
5.86	50	50	0	10	10	188
7.42	56	0	44	50	21.5	15
4.84	70	0	30	20	17	65

* The amount of oxygen present was at least 5.12 cc. per liter.

With the exception of the last two items, this table deals with one stock kept in a large aquarium in running water. The tests were made by selecting individuals at random and so give a fair representation of the reaction of the group. The increase in positive responses is not great but it is marked enough and constant enough to show that increasing the amount of oxygen present will affect the rheotactic reaction. One very significant fact is that there is no evidence of acclimatization in this or any subsequent test. The last two items show the same response with isopods kept in still water which gained its increased oxygen supply from the photosynthesis of *Amblistigium* moss.

If increasing the oxygen present in the water 5 cc. per liter caused an increase in the positive response, then increasing the concentration still more should cause a yet greater increase of the positive reaction. In order to test this, oxygen was bubbled through the aerating device previously described. The oxygen gave the following analysis; oxygen, 99 per cent, carbon dioxide trace, nitrogen 0.95 per cent. The results are listed in table 22.

The increase in the oxygen caused a decided increase in the positive reactions as well as in the general activity. Where isopods in the control crawled slowly these ran rapidly, often

TABLE 22
The effect of water having a saturated oxygen content, upon rheotaxis in adult pond Asell. Temperature 17°-21°C.

EXPERIMENT					CONTROL			
O ₂	RESPONSE		NUMBER OF TRIALS	TIME EXPOSED	O ₂	RESPONSE		NUMBER OF TRIALS
cc. per liter	per cent +	per cent -	per cent ±	days	cc. per liter	per cent +	per cent -	per cent ±
<i>I</i>								
1.25	32	28	40	0				
24.42	50	14	36	14				
24.42	48	16	36	14				
24.45	65	15	20	26				
<i>II</i>								
6.12	50	14	36	0	5.12	50	16	34
25.26	78	8	14	7	4.89	36	46	18
<i>III</i>								
1.53	38	28	34	0				
25.39	48	4	48	4	4.89	37	27	36
7.69	33	40	17	12				60

starting suddenly when no apparent stimulus was acting. Even when the rate of positive response was already high, as shown in the second part of the table, increasing the amount of oxygen present caused a large increase in the positive reaction. The third part of the table 22 shows the effect of allowing the oxygen in the water to escape gradually, and under these conditions the animals returned to a normal pond response while the amount of oxygen present was still much higher than that in their normal habitat. However it was very low in comparison with the amount of oxygen to which they had been previously exposed.

(2) *Juvenile mores.* Table 23 gives the effect of keeping juvenile pond Aselli in high oxygen for long periods of time. Other tests run for shorter times, give similar results but with less increase in positiveness. The right hand side of the table gives averages from the response during normal development and is placed here for comparison. Two things are brought out by the table. First, the pond isopods kept in a large amount of oxygen, develop not as normal pond mores but as stream Aselli, and second, that the time taken to acquire a given size is less when a larger amount of oxygen is present. The final results are especially interesting. At the age of 123 days the pond Aselli in high oxygen were 6 mm. long and gave a 72 per cent response. It took those in low oxygen 193 days to attain the same size and then they gave only a 38 per cent positive response. There is no evidence of a return to the response normal for pond isopods.

(3) *Response in straight current.* Again the tests with the continuous straight current support the results with the discontinuous circular one. The results of these trials are summarized in table 24. Isopods from all three methods of furnishing increased oxygen were used with similar results, and one is forced to the conclusion that increasing the amount of oxygen present in the water makes juvenile pond Aselli give a positive rheotactic response comparable with that given normally by stream isopods.

TABLE 23
The effect of high oxygen upon the rheotactic response of juvenile pond Asellid. Temperature 16°-23°C. (room variation)

O ₂ cc. per liter	LENGTH mm.	EXPERIMENT		NUMBER OF THALPS	APPROXIMATE AGE days	TIME IN HIGH O ₂ days	AVERAGE RESPONSE FROM STOCK IN LOW OXYGEN		AGE days
		per cent +	per cent -				AVERAGE O ₂ cc. per liter	SIZE mm.	
6.54	3	40	42	52	61	60	2.22	3	21
6.73	4	38	62	66	66	25	2.73	4	28
6.49	4	47	47	55	66	25			119
8.42	6	72	6	50	123	123	3.71	6	38
									193

TABLE 24
Pond Asellid from high oxygen in straight current. Current 1000-1100 cc. per minute

O ₂	TIME* BEFORE REVERSAL	POSITIVE PAN- RESPONSE	LENGTH OF REVERSAL mm.	TEMPERATURE (°C.)		REMARKS
				Trough	Aqua- rium	
cc. per liter				4	10	Aerating device for 10 days
8.51	2:38	55	26:58	10	18	moss 65 days
4.84	5:32	70	17:58	10	13	Run'g water 40 days
8.75	2:30	43	13:10	17	10	Run'g water 73 days
9.32	2:10 (after)	28	2:10	15	10	Run'g water 73 days
8.96	1:18 (after)	55	1:18	7	10	Run'g water 75 days
8.96	3:09 (after)	30	3:09	5	10	Run'g water 75 days
9.32	5:09	56	22:33	5	10	Run'g water 89 days
10.52	4:56		12:05	5	9	Run'g water 98 days
9.22	6:42	52	22:44	5	8	Run'g water 124 days
10.53	2:15 (after)		2:15	4	10	Run'g water 180 days
5.86	5:32	56	17:58	10	11	

* As before, the time is given in hours and minutes and the pan response in terms of the per cent of positiveness.

b. Caffein

Physiologically, caffein acts as a permanent stimulant. That is, there is no depressing after effect (Cushny, '10, p. 248). For this reason, experiments were run with pond isopods to find if such a stimulant would cause the same increase in the rheotactic reaction as that already produced by increasing the oxygen supply. For the experiments a solution of caffein in distilled water was made, saturated at room temperature, and this was added in small amounts to the ordinary tap water used in the experiments. The amount of caffein is shown in the table (25) in terms of cubic centimeters per liter of water.

The results of a number of trials are summarized in table 25. The first part compares the effects of different strengths of caffein solution upon the rheotactic response. For the time used, 25 cc. of the saturated caffein solution per liter of water proved to be most stimulating although the mortality was high. Part 2 gives a series of trials with a group of pond Aselli exposed to 10 cc. of caffein solution per liter of water. The positive response was increased over 30 per cent and continued high for over eighty hours. The subsequent decrease in the positive response is due to the fact that the isopods apparently became acclimated to the caffein.

In the third part is given the record of another set of trials with 10 cc. of caffein solution per liter of water. Under these conditions, the isopods showed a slightly higher positive response and gave the same slump in positiveness at about the same time. These experiments were run at the same time as those given in part 2 and the same control was used. Part 4 shows the effect of increasing the caffein after the animals had gone back to their normal reactions, through becoming acclimated to the strength of caffein used. The increase given must be due to the increased amount of caffein, because no other factors were acting. After the acclimatization to this amount of caffein occurred, a further increase served to kill the animals rather than to increase their positive reaction. Again as in the case of the saturated oxygen, the isopods are decidedly more active in all their movements so

TABLE 25
The effect of caffeine upon rheotaxis in adult pond Aselli. Temperature 17°-21°C.

EXPERIMENT				CONTROL					
O ₂ cc. per liter	CAFFEIN cc. per liter	RESPONSE		NUMBER OF TRIALS	TIME hours	O ₂ cc. per liter	RESPONSE		NUMBER OF TRIALS
		per cent +	per cent -				per cent +	per cent -	
5.04	0	29	32	50	0				
5.04	25	40	48	40	86				
5.29	22	38	40	50	94				
5.29	11	23	72	40	94				
	30	14	70	50	158				
II									
4.89	10	37	26	60	0	4.90	30	44	26
4.89	10	50	22	50	17	4.50	26	24	50
4.34	10	48	30	50	48				
4.45	10	54	28	50	83				
4.83	10	40	25	50	94				
4.15	10	27	32	50	166	3.75	33	27	40
									50

See control above

III

4.80	0	37	26	37	60	0
4.80	15	44	36	20	50	17
4.48	15	52	32	14	50	48
4.58	15	58	30	12	50	83
4.19	15	44	20	32	50	94
4.29	15	26	51	20	20	165

IV

4.21	10 and 15	26	44	30	130	$\frac{d_{cys}}{7}$
4.80	25	58	25	17	60	13
5.02	25	18	66	16	50	17
4.25	35	20	10	70	10*	23

* Others all dead.

that their general activity as well as their rheotactic response is affected.

c. Increase of temperature

It was shown in a previous section that decreasing the temperature caused a lowering of the positive response; theoretically then an increase of temperature should show reversed results. The first experiment to test the effects of increased temperature was carried on by keeping the animals near a hot electric plate. The second, by keeping the animals in an automatically regulated heating tank. The results listed in the third part of table 26 were obtained by keeping the isopods in a melting ice pack exposed to a warm room temperature.

The first part of the table shows that the effects produced by an increase of 6° operating for twelve hours, will persist for at least that much longer although the isopods are returned to the original temperature. The second set of trials indicates that the effect of continued high temperature is only temporary and the last three tests show that an increase of only 4° may cause a most decided increase of positiveness. The last experiments were upon stream *Aselli* during the breeding season.

Other experiments show that a sudden increase of 10° in temperature may cause either an increase or a decrease in the positive reaction, but if an increased response is still given after a 10° raise in temperature, more heat always results in a diminution of the positive response. Whether a 10° increase in temperature will cause an increase or decrease in the positivity, seems correlated with the general amount of activity of the isopods.

3. EFFECT* OF OXYGEN ON SIZE

The relation between rate of growth and the ultimate size of the isopods, with the amount of oxygen present in the water has been mentioned already, yet this subject is of enough importance to merit a short general treatment. Colton ('08, pp. 410-447) demonstrated for snails, that the size may be correlated with the general oxygen supply, but the exact amount of oxygen present

TABLE 26

The effect of raising the temperature upon rheotactic response

O ₂ cc. per liter	TEMPERATURE	RESPONSE			HOURS EXPOSED	NUMBER OF TRIALS
		per cent +	per cent -	per cent =		
4.89	19	20	24	50		50
	25	66	24	10	12	50
3.75	19	56	24	20	23	50
3.75	19	33	27	40	93	60

II						
6.15	18	42	36	22		50
	27	62	6	32	22	50
3.93	26	68	24	8	46	50
	26	40	12	18	70	50
4.55	27	42	18	40	121	50

III						
10.47	4	28	57	25	0	80
10.47	8	46	40	14	2	80
8.73	4	21	23	56	5	80

was not determined. It has already been stated that the stream isopods are on the average about 3 mm. larger than those found in ponds. One set of twenty pond males averaged 12.75 mm. in length; a set of twenty-eight pond females averaged 9.14 mm. The average of twenty-five stream isopods of each sex gives the males a length of 15.3 mm. and the females 12.2 mm.

During the course of the experiments, the difference in size of isopods reared in low and high oxygen was quite noticeable and those with the higher amount of oxygen, other things being equal, were of a larger size. Although only a few of these were measured, the measurements were entirely characteristic of the general effect and are shown in table 27, together with the average oxygen content and approximate age of the isopods measured.

Both sets of isopods were entirely comparable, being taken from the same stocks of animals. Both had plenty of food although perhaps the pond animals were better supplied. The

stream isopods were all kept in still water, the higher amount of oxygen being supplied by means of the aerating device. In the pond isopods, however, those from the higher oxygen were kept in running water so that they were in a medium free from their waste products, which other workers have found to cause a diminution of size (Colton, l. c.). The results indicate that the amount of oxygen present is one of the factors and probably a major one in causing the size difference between pond and stream Aselli.

TABLE 27
Effect of oxygen on size

<i>Stream isopods</i>	
LOW O ₂	HIGH O ₂
Age.....160 days	Age.....169 days
Average O ₂ , cc. per liter.....3.53	Average O ₂ , cc. per liter.....6.02
Average size, 20 isopods.....3.95 mm.	Average size, 9 isopods.....5.89 mm.
<i>Pond isopods</i>	
LOW O ₂	HIGH O ₂
Age.....116 days	Age.....123 days
Average O ₂ , cc. per liter.....2.48	Average O ₂ , cc. per liter.....7.09
Average size, 5 isopods.....4 mm.	Average size, 5 isopods.....6 mm.

4. REACTIONS TO GRADIENTS OF GASES

Earlier experiments had shown that isopods would collect in their optimum light or temperature conditions if subjected to a series of graduated changes in either of these conditions. Since their rheotactic activity is more dependent on the oxygen supply than upon either light or temperature, experiments were tried to determine whether or not the Aselli would respond to gradients of oxygen and carbon dioxide.

These experiments were run in galvanized iron boxes especially designed for the purpose. Each box was 50 x 30 x 7.5 cm. in dimensions. The bottom was covered with a layer of beeswax to give the isopods a better foothold for crawling. All the metal

parts were painted dead black. Two centimeters from each end a screen of brass wire netting was placed in order to keep the isopods from the current introducers. These introducers were of brass tubing in the form of a capital *T*. An even distribution of the current across the pan was assured by having the cross bar of the *T* punctured by eight equidistant holes, each 3 mm. in diameter. The water was withdrawn by a brass tube 2 cm. in diameter placed at the middle of the box with its lower side 4 cm. from the waxed bottom. This was also drilled with equidistant holes, which were guarded by wire screening to prevent the isopods from escaping.

Tap water was introduced at one end and the boiled or the high carbon dioxide water, as the case might be, was allowed to flow in at the other. The most striking results were obtained with a flow of 200 to 400 cc. per minute at each end of the pan. This current spread over the 10 cm. width of the pan, gave a flow of 5 to 10 cc. to each square centimeter of cross section, for each minute, and isopods do not react definitely to a flow of this strength.

With this device, a gradient of from 1.82 cc. of oxygen at one end of the pan to 8.14 cc. at the other, was obtained. For changes in salts see table 9 and discussion on p. 296. The half bound carbon dioxide was decreased 1 cc. per liter and the nitrogen content was lowered from 18 to 3 cc. per liter. In all cases a control was run in a box, the exact duplicate in every way of the experimental one. The only difference between the two sets of conditions was that in the control the same kind of water was introduced at both ends, the rate of flow being the same as in the experimental box. From five to ten isopods were used at a time and the experiments ran from one to six hours. Readings were taken at five, ten or fifteen minute intervals. In all cases the trials were made in very diffuse light or in total darkness. The readings were taken with a one candle power white light. The temperature was kept near enough that of the water from which the isopods were taken so that there was no temperature interference.

Table 28 gives the summary from eight trials made with isopods from water having an oxygen content of over 5 cc. per liter. The grand totals show a decided positive response to the tap water end, although the control animals are almost equally distributed between the two ends. This mass result was confirmed by transferring the introducers and thus gradually changing the amount of oxygen present at either end, and also by stirring the isopods back to the center. In all cases the final reaction was in favor of the tap water end. Generally this reaction was the result of a series of movements about the box during which the isopods went from one end to the other but in a few cases there was a definite turning back when the boiled water was encountered.

Table 29 shows the response of isopods kept in an oxygen supply of less than 3 cc. per liter. Even under these conditions they did not collect in the boiled water end of the gradient in greater numbers than in the tap water end, yet the response to the tap water was cut from 77 to 53.3 per cent of the total number of readings taken. This probably means that having been kept in low oxy-

TABLE 28
Stream isopods from high oxygen in an oxygen gradient

	EXPERIMENT				CONTROL		
	LOW	CENTER	HIGH	CURRENT	LOW	CENTER	HIGH
				cc. per min.			
O ₂ gradient cc. per liter.....	2.56	5.58	7.68	1200			
response.....	15	5	40		20	4	17
gradient.....	3.07	5.47	7.63	600			
response.....	6	2	122		53	9	68
response.....	20	21	87		no control		
gradient.....	1.82		8.14	200			
response.....	28	10	178		102	39	75
	87	22	388		169	23	169
	32	18	64		62	35	36
	9	4	73		37	168	45
Totals.....	197	82	932		434	278	410
Percentage of total trials.....	16	7	77		39	25	36

TABLE 29
Stream isopods from low oxygen in an oxygen gradient

	EXPERIMENT			CURRENT	CONTROL			O-OF CULTURE
	LOW	CENTER	HIGH		LOW	CENTER	HIGH	
				cc. per min.				cc. per liter
O ₂ gradient cc. per liter	3.07	5.47	7.63					
response.....	23	18	40	600	5	3	26	2.44
gradient.....	1.82		8.14					
response.....	26	9	151	400	41	6	139	2.12
	95	43	286	525	189	38	197	2.22
	80	26	71	525	65	43	82	2.56
	22	9	26	300	25	19	22	3.00
	42	38	96	300	39	86	51	2.00
gradient.....	3.24		11.00					
response.....	79	12	61	300	67	11	74	0.3
	17	30	28	300	77	11	11	2.44
Totals.....	384	185	759		508	217	605	
Percentage of total trials.....	29	14	57		38	17	45	

TABLE 30
Stream isopods in a carbon dioxide gradient
From water having less than 2 cc. per liter current 300 cc. per min.

	EXPERIMENT			CONTROL		
	HIGH	CENTER	LOW	HIGH	CENTER	LOW
CO ₂ gradient cc. per liter	24		3.5			
response.....	25	7	45	30	3	44
gradient.....	45		3			
response.....	28	2	40	17	29	34
gradient.....	59		3			
response.....	20	3	37	36	8	16
gradient.....	60		7			
response.....	7	0	36	23	4	15
gradient.....	80		20			
response.....	19	0	59	52	9	17
gradient.....	551		138			
response.....	5	0	72	38	6	40
Totals.....	104	12	289	196	59	166
Per cent of total number of trials.....	26	3	71	47	14	39

gen conditions, the isopods became more tolerant of low oxygen, although their optimum amount of oxygen was still higher than that furnished by the low end of the gradient.

The results with a carbon dioxide gradient are given in table 30. These trials show that stream isopods avoided an amount of carbon dioxide equivalent to the highest quantity found in nature, but that the avoiding is much more pronounced as the amount of carbon dioxide is increased. The result is, that although the higher amount of carbon dioxide diffuses against the current to the low end of the gradient, the isopods collect in an amount of carbon dioxide to which they are strongly negative when such a response is possible.

These gradient experiments show that the stream isopods tend to collect in the amount of oxygen or carbon dioxide to which they are accustomed. In nature this reaction would tend to keep the stream isopods from collecting in conditions that might affect their general state of metabolism, and in this way affect their power of resistance to the stream current.

5. SUMMARY OF EXPERIMENTAL RESULTS

The major experiments may best be classified on the basis of the result, of the materials used, upon the rheotactic response. Under this division they fall naturally into two groups; those that decrease and those that increase the positiveness of the rheotactic response. These may be summarized as follows:

A. Conditions that decrease the positive rheotactic response:

1. Low oxygen
2. Chloretone
3. Potassium cyanide
4. Low temperature
5. Sudden extreme increase of temperature
6. Carbon dioxide.
7. Starvation

To these may be added the life history effects as shown in the breeding season and the juvenile reactions.

B. Conditions that increase the positive rheotactic response:

1. Atmospheric saturation of oxygen
2. Complete saturation of oxygen
3. Caffein
4. Increase of temperature, if not too extreme

The rheotactic responses given by the isopods under different conditions are summarized in the following series of curves. These graphs are based upon all the experimental data at hand, and plot only the positive responses of the isopods. They necessarily show only two factors, namely, time and the per cent of

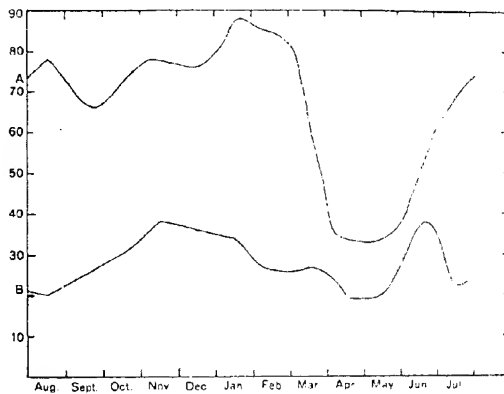


Fig. 5 Normal rheotactic response. A, Adult stream; B, Adult pond isopods

positive responses. The former is given by the abscissae and the latter by the ordinates. The other accessory data may be found by referring to the different tables.

Figure 5 shows the curve for the normal rheotactic response for adult stream (A) and pond (B) isopods. The general similarity of the two curves is noteworthy. The most striking difference, aside from the lower positive response of the isopods, is the different degree in which the breeding season affects the two mores.

The unbroken line (*A*) in figure 6 gives the effect of a decreased oxygen supply upon adult stream Aselli. The response consists of four periods. First there is the lessening of the positive response which is followed by partial acclimatization. This is followed by the breeding season after which the isopods are either more vigorous or else are more capable of becoming acclimated than before and so give a stronger positive response. The broken lines (*B*) show the effect produced by keeping the pond Aselli in tap water, the steeper curve being plotted for younger animals. In neither case is there any sign of acclimatization.

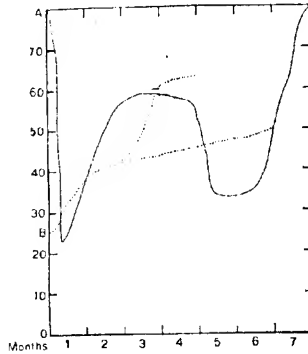


Fig. 6 Adult isopods in reversed oxygen supply. *A*, Adult stream; *B*, Adult pond isopods.

The broken line (*B*) in figure 7 gives the results produced by a low oxygen supply, upon the rheotactic response during the breeding season. In general there is first an increase of the positive response, followed by a decided reversal. The unbroken line (*A*) shows the effect of an increased carbon dioxide supply. The effect of the decreased oxygen is probably due to its action first, upon the germinal glands, causing a cessation of their activity, which gives more energy for other activities. This is followed by the direct depressing effect of the low oxygen upon the tissues themselves. The results so far obtained with carbon dioxide may be explained by its general narcotic action.

The solid lines in figure 8 give the effect of low (S_2) and high (S_1) oxygen content upon the rheotactic reaction of juvenile-stream Aselli. The rheotactic reactions are too indefinite during the first month to be considered. The second month shows the same state of development in each environment but from that time on the responses are entirely different. It is especially noteworthy, that the isopods kept in a low supply of oxygen throughout their lives, did not become strongly positive even after the breeding season, which came in their fifth month. That is, they did not show the same capacity for regulating, given by adults under the same conditions. The broken lines give the reactions of isopod

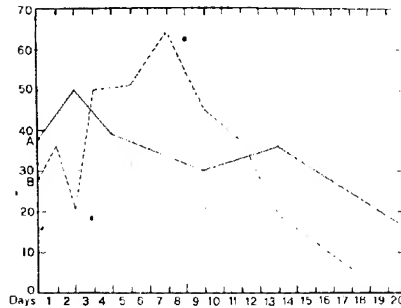


Fig. 7 The effect of increased carbon dioxide and decreased oxygen upon the rheotactic reaction during the breeding season. A, carbon dioxide; B, oxygen.

of pond parentage (P_1) in high, (P_2) in low oxygen supply, and it is evident that their rheotactic reactions do not depend on their ancestry but on their environment.

Figure 9 compares the results of different depressing agents at the concentrations used. Apparently a rapid decrease in temperature (A) is most effective and most transitory. Carbon dioxide (C) from 200 to 300 cc. per liter seems to be second in both these qualities. The isopods become slowly acclimated to chlorethane (B) at the strength used, but show no such tendency with the potassium cyanide (D) in the concentration used. Starvation (E) also acts as a continued depressant.

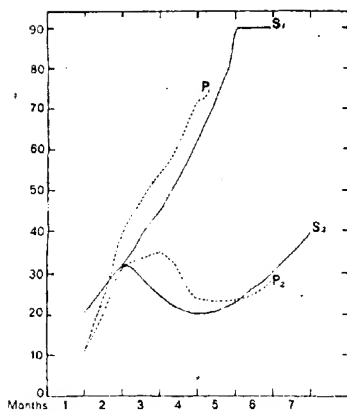


Fig. 8 Rheotaxis during isopod development in different amounts of oxygen. S_1 , Stream isopods in high oxygen, S_2 , in low oxygen; P_1 , pond isopods in high oxygen, P_2 , in low oxygen.

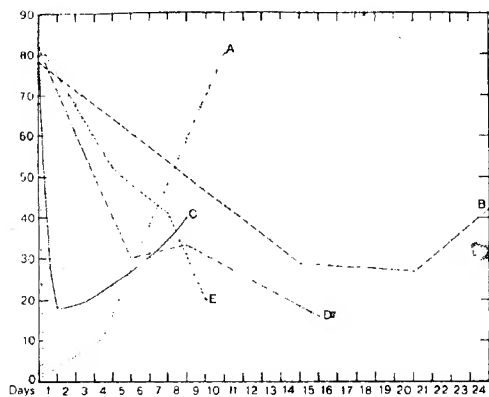


Fig. 9 The effect of depressing agents upon rheotaxis. A, low temperature; B, chloretone 1/200 per cent solution; C, carbon dioxide, 200-300 cc. per liter; D, KCN, n/100,000; E, Starvation.

Figure 10 gives the curve for the effects produced by stimulating agents. Two distinct results were obtained with water saturated with oxygen (*B, D*). In one case (*B*) the isopods were already giving an increased positive response due to the relatively high oxygen content of the water. In both cases, however, the increased positiveness was maintained until death resulted. The isopods, however, become acclimated to both a higher temperature (*A*) and to a supply of caffeine (*C*). When once acclimated to

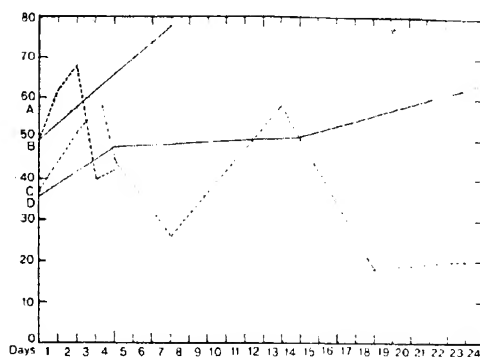


Fig. 10 The effect of stimulating agents upon rheotaxis. *A*, Increased temperature, about ten degrees; *B, D*, saturated oxygen, 25 cc. per liter; *C*, caffeine, 10-25 cc. saturated solution per liter.

the latter, a further increase up to the point of toxicity caused increases in the positive response.

The experiments have also shown that the size of the isopods and their rate of growth are correlated with the oxygen supply. Also that when subjected to a gradient of different concentrations of oxygen or carbon dioxide, *Aselli* will collect in the concentration nearest that to which they are accustomed.

IV. DISCUSSION

In presenting these results the writer realizes fully the attending imperfections and the need of more work along some lines. But in order to do further work with this material, in this field, in an intelligent manner, these general relationships had to be first blocked out. It is the author's intention to follow this paper with a study of the effect of the same conditions upon another characteristic isopod reaction, and to further analyze the interesting relations shown during the breeding season.

The principles upon which this paper is based have been successfully worked out (under the author's direction) with amphipods by Mr. W. J. Saunders in an unpublished master's thesis and with amphipods and planarians by graduate students doing course work in experimental animal behavior. The work of these students has shown that the rheotactic reactions of both amphipods and planarians are comparable with those of pond and stream isopods and may be controlled by the same external factors.

In the summary of the experimental results just given, the basis for classification was made the positiveness of the isopods in their rheotactic responses, but if the heading were rather the effect of these conditions upon the metabolic state of the organism, the summary would not need to be changed. For all the conditions that have been found to cause a decrease in the positive reaction are known to depress the rate of animal metabolism (Child, *l.c.*, p. 173). Low oxygen (Haldane and Smith, '97, p. 242) potassium cyanide (Ceppert, '99, p. 208) and high carbon dioxide (Cushny, '10, p. 587) do this by directly decreasing the oxidations. Chloroform belongs to the general group of anaesthetics that are known to have a depressing effect upon certain of the fundamental metabolic reactions (Child, *l.c.*, p. 173; Cushny, *l.c.*, p. 195). The decrease in activity due to low temperature is a well known phenomenon in both animals and plants, as is also the depressing effect of an increase of temperature above the optimum for life relations, while starvation decreases metabolism by removing the material to be oxidized and so gives the same results from the other side of the equation. On the other hand, the rate of metab-

olism increases with the increase of the oxygen dissolved in water (Lingle '02, p. 83; Martin, '06, p. 303; Loeb, '06, p. 95). Then, too, Piéron ('08, pp. 1020 and 1061) measured the amount of oxygen present at different times in sea water and found that Actinians expand and retract their tentacles as the oxygen tension of the water increases and decreases. These observations were confirmed by experiments in aquaria, but Bohn ('08, p. 1163) questions Piéron's interpretation of his results and attributes these rhythms to much more complex factors. An increase in temperature increases metabolism providing, of course, the increase is not too rapid or too great. Caffein is known to have a permanent stimulating effect (Cushny, l.c., p. 248).

From this evidence, the rheotactic reaction in isopods must depend upon the metabolic state of the animals. All the observed facts concerning their response in nature support this view, except that in young animals the rate of metabolic reaction is higher than in the older ones. Yet the juvenile isopods give either no rheotactic reaction or are very indifferent to the current. But with these young isopods, conditions that favor a high metabolic rate cause positive reactions to appear sooner and to be a great deal stronger than in those animals kept in conditions that depress metabolism. Then, too, the clinging reaction is strong in these young isopods and even under the most favorable conditions, they do not move about rapidly. Since the positive rheotactic response seems correlated with the degree of motile activity of the isopods, this tendency to cling in one place would account for the apparent discrepancy in the lack of positiveness in these juvenile Aselli.

In nature the complex of conditions found in ponds furnishes isopods that give a low positive rheotactic response, while stream conditions produce the opposite result. The amount of oxygen in these two habitats varies greatly, as has already been pointed out, and this variation appears sufficient to account in a large measure for the difference in the reactions. Of course the fact that the pond water usually contains more organic waste products than are found in the streams, may help to cause the difference in the reactions. But this does not seem to be the important

factor since pond isopods kept in well aerated water containing a large amount of waste products gave the same increase in the positive reaction, as when running tap water was used. Also the cause of difference in response in isopods from the two habitats cannot be due to the mechanical stimulation of the current for experiments with both stocks show that this cannot be true. Pond Aselli kept in still water but in high oxygen, increase in positiveness, while stream Aselli under the same conditions maintain their high percentage of positive responses. The isopods which gave the reactions in table 1 had been in still water all their life and yet gave a positive response of over 89 per cent. From these considerations it appears that oxygen is either the most important environmental factor in determining the rheotactic response or it is the best single factor index of the effect of the complete environment upon this reaction.

Through the tables one can see evidences of irregularity in the response of the isopods from the same conditions. This irregularity was due to two causes. First, the error of the method, which has been shown to be about 5 per cent. This error is due, in part, to the tendency of the isopods to keep on going in the way they may be headed. This would account for an isopod going positive nine times and negative the tenth. The second cause of the irregularity is the fact that an 80 per cent positive response on the part of five isopods may mean that four of them went positive every time while the fifth was entirely negative. At first sight this would appear to be a serious objection to the plan of selecting the animals to be tested at random from the stock under consideration. Conceivably one might pick five isopods that would all give a response opposite to that of the general culture. In practice however this has not occurred and the large number of trials is a sufficient safeguard against such a source of error. However in the later experiments every animal used, both in the control and in the experiment, was tested and more uniform results were obtained in this way.

The question at once arises as to why one member of the stock under the same external conditions as the others should give a different rheotactic response. One of the reasons is the state of

the isopod, regarding the time distance from the moulting period. The exact bearing of the moulting cycle upon rheotaxis has not yet been worked out, but evidence is accumulating to show that in stream isopods, the moulting period and the time immediately following it are characterized by an indefinite or at any rate a weak rheotactic response.

Regarding the permanence of the modifications produced, it has been repeatedly mentioned, that in pond isopods kept in high oxygen, there was no reversal to the normal pond response, although in two cases these experiments ran for over six months. On the other hand, the stream isopods seemed to possess a power of acclimatization, and a return to the normal stream positiveness in a fairly low oxygen content. However, in the case of the isopods reared entirely in low oxygen, this increase in positiveness was much less marked. Apparently if the stock could have been carried through a few more generations the response would have been entirely that of pond isopods. Hence it would appear that the reactions, with which this paper is dealing, are distinctly dependent upon the environment for their continuance. On the other hand the taxonomic differences, as the number of spines on the propodus of the first thoracic appendage, show the same variation in both habitats. That is, the taxonomic differences are inheritable characteristics of the species, and are not dependent upon external conditions, while the behavior characters here studied are almost independent of heredity.

The size of the isopods has been shown to be correlated with the amount of oxygen in the water and size is often used in defining taxonomic species. So here we have one structural element that does depend directly upon the environment. This bears out the statement of Shelford ('11 a, p. 593) that animal behavior is usually plastic while animal structure is only slightly plastic. Since aside from the size, the taxonomic characters of isopods from the two habitats are the same, the animals cannot be referred to as a stream 'form' as contrasted with a pond 'form,' because in its general usage form is applied to a morphological entity. However, the term 'mores' as used in general in the Concilium Bibliographicum, and as specifically defined by Shelford ('11, p. 30)

does exactly express the difference between the pond and stream isopods. Thus we are dealing with the pond *mores* and the stream *mores* of *Asellus communis*, which depend on environmental rather than on hereditary differences for their distinctive features.

In the isopods used, these two mores occur in the same taxonomic species, but in the amphipods, Mr. Saunders (l.c.) found a different species in the ponds from that in the streams. This means that with the amphipods studied the natural mores would run parallel with the taxonomic species. But the pond mores could be transferred directly into stream mores without affecting their structural characters. Thus the mores or 'ecological species' are independent of *taxonomic* species, and a single ecological species may be composed of half a dozen taxonomic species or of only a fraction of one.

The general conclusion to be drawn from this series of experiments is that in the isopod, *Asellus communis*, the rheotactic reaction is dependent upon the metabolic state of the animal for its degree of positiveness and that the natural or experimental conditions which affect the metabolic state of the animal, change its rheotactic response. That is to say, the rheotactic reaction is here an expression of the metabolic condition (physiological state) of the isopod and may be controlled by those factors known to control animal metabolism.

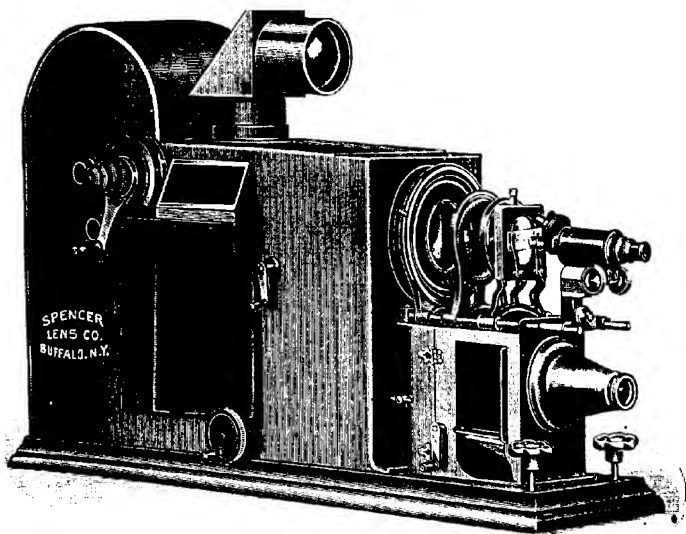
It is a pleasure to acknowledge my indebtedness to Dr. C. M. Child for his many helpful criticisms but my best thanks are due to Dr. V. E. Shelford, who first suggested this problem and under whose direction the work has been done.

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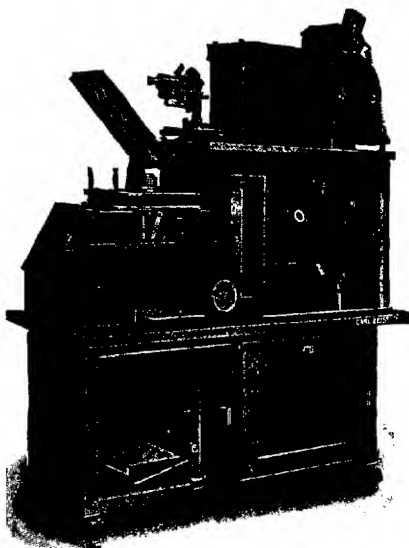
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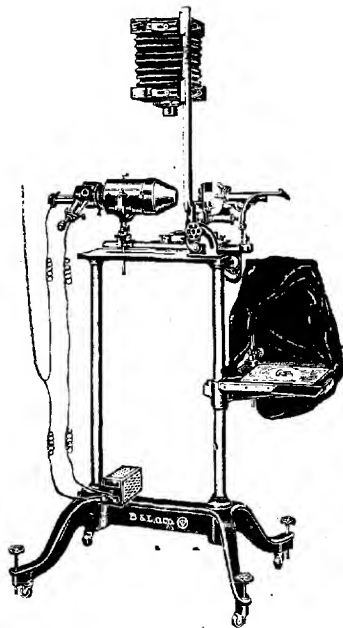
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STUDIES ON CHROMOSOMES

VIII. OBSERVATIONS ON THE MATURATION-PHENOMENA IN CERTAIN HEMIPTERA AND OTHER FORMS, WITH CONSIDERATIONS ON SYNAPSIS AND REDUCTION

EDMUND B. WILSON

From the Department of Zoology, Columbia University

NINE PLATES

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INTRODUCTION

In this paper are described observations on certain phases of the maturation-process in *Oncopeltus fasciatus* (Dall.); *Lygaeus bicrucis* (Say), and some other Hemiptera, together with the results of a comparison of these species with some other insects, with *Tomopteris* and with *Batrachoseps*. It was my original object to clear up the relations of the sex-chromosomes in *Oncopeltus* and to trace as completely as possible their history in the maturation-process; but in doing this it was found necessary to take into consideration many other features of the spermatogenesis, and I will take this opportunity to present some conclusions based on a broader study of these problems on which I have long been engaged.

In respect to the sex-chromosomes, *Oncopeltus* is of especial interest because it stands on the border line between species in which the *X*- and *Y*-chromosomes are visibly unequal in size, and in which a corresponding visible difference appears between the diploid chromosome-groups of the two sexes, and those in which such sexual differences can not be seen.¹ In my fourth 'Study' ('09 a) *Oncopeltus* was classed with *Nezara hilaris* as an example of the latter class of cases; and much theoretic importance has been ascribed to both these forms as indicating the possibility or probability that the spermatozoa are really sexually dimorphic even when no visible evidence of this is shown by the chromosomes. In my seventh 'Study' ('11 a)) I showed, contrary to my original account, that a dimorphism of the spermatid-nuclei is in fact visible in *Nezara*; but in regard to *Oncopeltus* judgment was reserved as I was still baffled by apparently contradictory data. I am now in a position to clear up these

¹ The first account of *Oncopeltus* was given by Montgomery ('01), who described the sex-chromosomes ('chromatin-nucleoli') as of equal size in the male, and found that they remain always separate, without fusing at the time of general synapsis, and divide separately in the first spermatocyte-division. Subsequently ('06) he added that these chromosomes (now called 'diplosomes') conjugate to form a bivalent after the first division, and undergo disjunction in the second division. In my fourth 'study' ('09 a) I briefly confirmed these accounts, and stated that the female diploid chromosome-groups are not to be distinguished by the eye from the male.

contradictions and to announce a definite result. *Oncopeltus* is indeed a case in which the X- and Y-chromosomes are very often sensibly equal in size, and in which the sexual differences of the diploid groups are too elusive to be certainly distinguished by the eye. These differences, nevertheless, almost certainly exist. In certain individuals a distinct size-difference between X and Y is clearly evident in a large percentage of the cells at every stage of the spermatogenesis; and even in individuals where they usually appear equal, inequality is unmistakably seen in a small percentage of the cells. Aside from this, the close similarity—almost identity—between *Oncopeltus* and *Lygaeus bieruei* in all other features of the spermatogenesis makes it extremely probable that the same essential relations of the chromosomes to sex exist in both, though they are only clearly obvious in *Lygaeus*.

Like many other insects of this order, *Lygaeus* and *Oncopeltus* are distinctly unfavorable objects for the direct study of synapsis and the reduction-division—indeed the problem of synapsis seems to be practically insoluble in these particular forms. They nevertheless present some very interesting features for comparison with other forms. In the first place, the history of the sex-chromosomes may here be traced with almost unique clearness. They may be identified at a very early pre-synaptic period, and followed thence as individual bodies through every later stage up to the time of their final delivery to the spermatid-nuclei. Every step may be followed in their conjugation and subsequent disjunction without any intervening process of fusion. In case of these particular chromosomes, therefore, I consider synapsis and disjunction to be indisputable facts. It is far otherwise with the ordinary chromosomes or 'autosomes.' It is extremely difficult to gain any clear idea of their behavior in the synaptic period, and I fear quite impossible to trace them individually through the growth-period. On the other hand, their behavior in the pre-synaptic period and in the maturation-prophases exhibits some very interesting features when compared with other forms in which the process of synapsis and its sequel are more accessible to observation. In making such a comparison I have been fortunate in the opportunity to make use of some remarkably fine

preparations of other investigators, to whom I am under great obligations. To Professor McClung I owe the loan of a beautiful series of orthopteran preparations, especially of *Phrynotettix*, *Mermiria*, *Chortophaga* and *Achurum*, which display on a larger scale some of the same phenomena seen in the pre-synaptic stages of the Hemiptera, and leave no doubt of the close parallel between the two groups in this regard. Even more, however, I am indebted to Dr. and Madame Schreiner, and to Professor Janssens, for some of their admirable original preparations of *Tomopteris* and *Batrachoseps*, which have enabled me to make a prolonged study of the phenomena of synapsis in these classical objects. In particular, two magnificent slides of *Batrachoseps* by Janssens demonstrate both the complete seriation of the stages and the finest details of the nuclear structures with incomparable clearness. Though I have also made many preparations of this form, as well as of *Plethodon* and other Amphibia, I must admit my failure to equal in all respects the standard set by the slides of Janssens. A close comparison of these various preparations has more than ever impressed me with the futility of attempting the study of these problems with material that is unfavorable for the purpose, or with preparations that in any respect fall short of the highest standard of technical excellence. Nothing is more certain than that different objects differ enormously in the clearness with which the relations are displayed, and in their reaction to fixing and staining reagents. Had this been more generally recognized, many erroneous conclusions and some ill-considered criticism might have been avoided.

Through the study of *Batrachoseps* and *Tomopteris* I have finally been convinced—for the first time, I must confess, as far as the autosomes are concerned—(1) that synapsis, or the conjugation of chromosomes two by two² is a fact, and (2) that in these ani-

² A number of writers have suggested that the term synapsis, as here employed, should be abandoned in favor of some less ambiguous word (such as Haecker's term 'syndesis') because it has so frequently been applied to the contraction-figure ('synizesis' of McClung). I am, however, in favor of the retention of the word, for the ambiguity has arisen simply through a misunderstanding of Moore's meaning. He applied the term 'synaptic phase,' or 'synapsis,' to the series of changes following the last diploid division (during the 'rest of transformation')

mals (perhaps also in the Orthoptera) the conjugation is a side by side union, or paräsynapsis. On the other hand, the evidence of a 'reduction-division' in the ordinary use of the term—i.e., the disjunction of the same chromosomes that unite in synapsis—seems to me to be far short of a demonstration. In these forms synapsis is followed by a union so intimate that no adequate evidence of duality can for a time be seen in the resulting bivalents. I do not for this reason argue against the conception of the reduction-division. On the contrary, I shall offer new considerations in favor of this conception in a somewhat modified form; but in case of the autosomes it must for the present rest mainly upon indirect evidence. In this respect the autosomes differ notably from the sex-chromosomes, at least in the male sex; and this difference may be of significance for some of the most interesting phenomena of sex-heredity.

in the course of which the apparent number of chromosomes is reduced to one-half. "There are thus, after the rest of transformation, only one half as many chromosomes, i. e., separate chromatin-masses, as there were before, and the halving of their number, being brought about while the nuclei are still at rest, is to that extent comparable to what is now known to go forward during the maturation of the reproductive elements of plants. I therefore propose the term Synaptic Phase (from *συνάπτω*, to fuse together) to denote the period at which this most important change appears in the morphological character of reproductive cells" ('95, p. 287. In subsequent pages the phrase 'synaptic phase' is often shortened to 'synapsis' in the same sense). This 'most important change' is obviously the halving of the number of chromosomes; and nowhere in his paper is the word applied to the contraction-figure, though the latter is stated to be "characteristic of this particular phase in the spermatogo- and ovogenesis of a great variety of animal forms" (p. 305). Though there was, perhaps, some obscurity in his original use of the word, all doubt as to Moore's meaning is removed in a later paper, published jointly with Farmer ('05), where synapsis is precisely defined as "that series of events which are concerned in causing the temporary union in pairs of pre-meiotic chromosomes" (p. 490). The fact that so many later writers have misapplied it should not debar us from the continued use of so convenient and appropriate a term—one that seems particularly fitting if it be a fact, as a number of excellent observers have concluded, that synapsis is followed by actual fusion. I can discover no reason why McClung's term 'synizesis' should not be generally employed for the contraction-figure, as it already is by most American writers.

I. THE MATURATION-DIVISIONS IN *ONCOPELTUS* AND *LYGAEUS* WITH REFERENCE TO THE SEX-CHROMOSOMES

In *Oncopeltus* the diploid number of chromosomes is sixteen in both sexes (figs. 1 to 5, photos, 1, 2) in *Lygaeus* fourteen (fig. 6), and the second spermatocyte-division shows half these numbers that is, eight in the former case, seven in the latter. The first division shows in each case one more than the haploid number, owing to the fact, repeatedly described heretofore in other Hemiptera, that in the first division the *X*- and *Y*-chromosomes divide as separate univalents, while in the second they are united to form a bivalent. In both *Oncopeltus* and *Lygaeus* these chromosomes conjugate in the final anaphase of the first division just as the cell is about to divide.

1. The diploid chromosome-groups

The spermatogonial and oogonial divisions require but brief description since they present no striking features and the size-differences are but slightly marked. In *Lygaeus bicrucis* (fig. 6) the fourteen chromosomes are in the main similar to those of *L. turcicus* as described in my first and third 'Studies' ('05, '06) though the *Y*-chromosome is relatively smaller in the latter species. The *X*-chromosome can not be identified by the eye, but must be at least twice the size of the *Y*-chromosome, as indicated by the maturation-divisions and by the spermatogonial groups themselves. Unfortunately my material of this species does not show a single good equatorial plate in the female; but the relations are here no doubt the same as in *L. turcicus*.

In *Oncopeltus*, of which I have abundant material of both sexes, the size-differences of the chromosomes are even less marked than in *Lygaeus*, and it is impossible to identify pairs of different sizes. Careful study fails to reveal any differences between the diploid groups of the two sexes that are sufficiently marked or constant to give any certain result (figs. 1 to 5). In the male one chromosome not infrequently is somewhat smaller than the others (fig. 2), and this may be the *Y*-chromosome; but very often this is not evident, even in other spermatogonia from the same cyst (figs. 1, 3). As will be shown beyond, the *X*- and *Y*-chromosomes

are often somewhat unequal in later stages; but this, too, is inconstant. *Oncopeltus* is in fact, therefore, a form in which the sexual differences of the chromosome-groups are too slight or too elusive to be distinguished by the eye. It is, however, perfectly certain that an *XY*-pair is present, the members of which show all the characteristic peculiarities of behavior that characterize these chromosomes in other forms.

2. *The first spermatocyte-division*

The maturation-divisions are shown with remarkable clearness in *Oncopeltus* and *Lygaeus*—indeed, either of them might be taken as a model of those Hemiptera in which a simple *XY*-pair is present. The following account applies primarily to *Oncopeltus*, *Lygaeus* being described only by way of comparison.

In the first division appear nine separate chromosomes in a grouping of remarkable constancy. Seven of the nine bivalents are grouped in an irregular ring, near the center of which lie the univalent *X*- and *Y*-chromosomes, side by side but not in contact (figs. 8, 9, 11, photo. 3). The constancy of this grouping appears from the following data. Two hundred clear polar views, taken at random, did not show a single case of more than nine chromosomes; plus variations of number in this division (such as are occasionally seen in many species) must therefore be very rare—indeed, I have never seen such a case.³ Of the two hundred cases, one hundred and seventy-two showed the grouping just described. In the remaining twenty-eight the deviations were unimportant; the ring may show a gap at one side (figs. 10, 12), one of the bivalents may lie inside it (fig. 10), or (rarely) one or both the sex-chromosomes may lie in the ring (fig. 13). In only two cases did the sex-chromosomes not lie side by side; in these, they were separated by one bivalent (fig. 13).

The size-relations alone sufficiently indicate that the two small central chromosomes are the univalent sex-chromosomes (a fact

³ Apparent minus deviations are of course common, but are disregarded because evidently due in most (all?) cases to the fact that one or more chromosomes lie outside the plane of section.

fully established by study of the growth-period and the pro-phases); for, were one or both bivalent, the spermatogonial groups should show one or two corresponding pairs, which is not the case. In *Lygaeus* (fig. 7) the grouping is the same, but only six bivalents are present, and the sex-chromosomes are conspicuously unequal in size.

The composition of these chromosomes is better seen in smears than in sections, and better in *Protenor* (described beyond) than in either of these forms. In sections, side views of the full metaphases (figs. 14 to 17, photo. 4) usually show all of the chromosomes as simple dumb-bell figures, though indications of a quadripartite form sometimes appear. In smears the *bivalents* are often seen to be quadripartite, owing to the presence of a longitudinal split in addition to the transverse constriction; but this never appears in the univalents. All the chromosomes alike divide 'transversely'—that is, across the constriction of the dumb-bell. In case of the bivalents, therefore, the early anaphase-chromosomes are double bodies, while the sex-chromosomes are single, but this contrast only appears clearly in smears, owing to the close union of the two halves. In this respect the relations are less clearly seen in these forms than in some others, such as *Anax*, where the anaphase-chromosomes are clearly double (*cf.* Lefevre and McGill, '08), or *Aprophora*, where the same condition is conspicuously shown (Stevens, '06).

The anaphases are of particular interest because, as has been mentioned, a conjugation between the X- and Y-chromosomes takes place in the later stages. As the division begins and the daughter-chromosomes are separating, a marked contrast in form often appears between the sex-chromosomes and the autosomes (figs. 19 to 21). The latter are more or less extended transversely and often show a slight constriction, thus giving evidence of their double nature, which is accentuated by the very conspicuous double fibres by which they are connected. The latter are so thick, and stain so deeply, as to appear as if spun out from the chromosomes themselves (as has been noted by other observers). On the other hand, the sex-chromosomes do not show such a constriction, remaining nearly circular in outline, while the connect-

ing fibers are much less conspicuous, and often appear single. Up to the middle anaphases the sex-chromosomes remain always separate (figs. 18, 19). In the later anaphases all the chromosomes draw more closely together, and often come more or less into contact, though without losing their original grouping; but in case of the autosomes the contact is but casual and temporary, while the sex-chromosomes become definitely attached to each other to form a dumb-bell shaped body at the center of the group (figs. 19, 21). By this process the total number of separate chromatin-elements is reduced from nine to eight (the haploid number). In *Lygaeus* the process takes place in exactly the same way and may be seen with equal clearness, both in polar views and in side-views. In both species polar views of the final anaphases show that the chromosomes, save for their more crowded condition, have retained the same grouping as in the metaphase (figs. 24 to 29), the *XY*-bivalent being at the center, surrounded by the other chromosomes in the form of a ring. These facts are seen so clearly and in so great a number of cases as to remove every doubt that *in these species the conjugation between X and Y regularly takes place at the poles of the spindle before the first maturation-division has been completed.*⁴

In *Lygaeus* the *XY*-bivalent thus formed is readily distinguishable by the inequality of its two components (figs. 28, 29, 46). In *Oncopeltus* it is often not thus marked but its identity is no less certainly revealed in another way. As the figures show, the autosomes still show but slight indication of a transverse constriction, and can hardly be described as dumb-bell shaped until a later period. The *XY*-bivalent, on the other hand, is invariably deeply constricted, so as to have a conspicuously dumb-bell shape, and it often still appears like two chromosomes that are merely in contact. This characteristic difference persists throughout the entire interkinesis, and is still perfectly obvious in the ensuing metaphase of the second division.

⁴ I described and figured this process in the case of *Coenus* in my first 'Study' ('05 b) but did not recognize its constancy. I now incline to think that it will be found to occur in the same way in many other forms.

3. *The interkinesis*

The interkinesis has hitherto been very briefly treated by myself and other observers of the insects, because in most species the chromosomes are so closely crowded at this time as to preclude accurate study. *Lygaeus* (at least in my material) is no exception to this, but *Oncopeltus* fortunately shows every stage of the interkinesis with remarkable clearness. There is no 'resting stage' between the two divisions, no nuclear vacuole is formed, and both the chromosomes and the centrioles retain their individuality throughout.

At the moment when the equatorial furrow has appeared and the conjugation of *X* and *Y* has taken place, the centrioles are already rather far apart, and still lie at some distance from the chromosome-group (fig. 22). All the achromatic elements are now so delicate that it is difficult to make sure of the exact structure; but it is certain that each centriole is surrounded by a small, but very distinct aster, and the two *seem* to be connected by a delicate central spindle. As the cell divides several other changes take place. The chromosomes, without otherwise changing their grouping become still more crowded together, and thus become massed in a nearly flat plate, while the centrioles move still farther apart (fig. 23). Shortly after the division these relations are unchanged save that the centrioles are still farther separated and lie nearly on opposite sides of the chromosome-group. The asters are still present, and between them lies a rather large, irregularly spindle-shaped area. It is difficult to say whether this should be regarded as an actual spindle; but delicate fibrillae often may be seen extending into it from the poles.

The chromosome-group lies somewhat excentrically within this area in the form of an irregular flattened plate. In side-view (fig. 30) it is usually impossible to distinguish more than a few of the chromosomes. In face view also, the crowding is often so great that the grouping can not be exactly made out. Here and there, however, it is evident that the original grouping has not been lost, and occasionally plates are to be found in which every chromosome may be clearly seen (figs. 31, 32). Study of such

cases makes it certain that no fusion or process of disintegration has taken place, nor is any evidence of a nuclear vacuole to be seen. The chromosomes still retain the same form as in the preceding anaphases, the XY-bivalent lying near the center, and still very clearly distinguished by its markedly bipartite form. A very characteristic feature of this stage is the massing of mitochondrial granules on one side of the spindle-area as seen in side-view (figs. 30, 31). This results from the fact that during the division the chondriosomes are mainly massed around the spindle and do not extend to any great extent into the polar areas (fig. 22). After completion of the division therefore the chondriosomes still lie mainly on the side of the chromosome-plate. In a general way this relation persists throughout the interkinesis. In the condition just described the cells remain until the prophases of the second division. It is probable that the interkinesis is of rather brief duration, because in some cysts all stages may be found between the closing anaphases of the first division and full metaphases of the second. Cysts may, however, be found in which practically all of the cells are in the condition described; from which it may be inferred that a brief pause follows the completion of the first division.

4. *The second spermatocyte-division*

The prophases of the second division, which follow directly upon the stage just described, are marked by a resumption of activity on the part of the astral systems, which rapidly increase in development while a definite spindle is formed between them. As this takes place, the chromosomes spread further apart and take up a position at the equator of the spindle *in the same grouping as before*. It is rather difficult to follow this change completely, as these stages are not very abundant in my preparations, and are almost always seen in oblique view. It is, however, certain that a double movement of the chromosomes takes place, involving (1) a rotation of each chromosome through about 90 degrees, so as to assume a position with its long axis parallel to the axis of the spindle, and (2) a virtual rotation of the entire group, so as

to lie at right angles to the spindle. I am uncertain whether the latter movement is a simple rotation of the group as a whole, or whether the relative position of the individual chromosomes changes more or less as they spread apart. It is certain, however, that when the metaphase has been attained (figs. 34-37, photo. 4) the chromosomes have the same general grouping as in the final anaphases of the first division or in the interkinesis, save that they are less crowded. As before, the XY-bivalent lies near the center, surrounded by the seven other chromosomes, very often arranged in an irregular ring, though this is somewhat variable.

It seems probable from the facts just described that in these animals the general grouping of the chromosomes is determined in the prophases of the first spermatocyte-division. Already at this time the X- and Y-chromosomes are brought into position for their ensuing conjugation; and their topographical relation to the autosomes remains thenceforward unchanged until their final delivery to the spermatid-nuclei. In this respect these species agree with such forms as *Fitchia* or *Rocconota* among the reduvioids (Payne, '09) and differ from the coreids and other forms in which a marked change of grouping occurs after the first division. I conclude, further, that neither the chromosomes nor the centrioles lose their identity in the period between the first and second divisions, and that a complete relation of continuity exists between the two generations of spermatocytes in this respect.

In side-views of the second metaphase the XY-bivalent is still almost always distinguishable from the other chromosomes by its deeply constricted dumb-bell shape (figs. 36, 37, 42, 43); and in correlation with this, this element is apparently always the first to divide, its two components having often completely separated before the others have even become deeply constricted (figs. 38 to 41, 44, 46, photo. 6). This precocious division of the XY-bivalent is a very common phenomenon among the Hemiptera (as I have heretofore described). It is obviously due to the comparatively loose union of X and Y after their conjugation, so that they yield more readily to the poleward force (whatever it may be) that operates during the division.

The later stages of this division have been so often described as to call for no further account. The final result is that the spermatid-nuclei receive the haploid number of chromosomes—seven in *Lygaeus*, eight in *Oncopeltus*—half the nuclei in each case receiving X and half Y. The facts seen so clearly in both these species remove every possible doubt that the X- and Y-chromosomes which thus enter the spermatid-nuclei are the same individual chromosomes that conjugate at the end of the first division and persist throughout the interkinesis to disjoin in the course of the second division. It is of course possible that some exchange of material may take place between them during the brief period of their association. Of this, however, there is no evidence; and it is certain that their individual boundaries are not lost to view, and that not even an apparent fusion takes place at this period or any earlier one.

5. *The size-relations of the sex-chromosomes in Oncopeltus*

In my first examination of this species my attention was given mainly to some excellent preparations from two individual males (designated by the numbers 711 and 712) in which the X- and Y-chromosomes appear equal in a large majority of the nuclei. The facts in *Nezara hilaris* (Wilson, '11 a) led me to extend the examination to other individuals of *Oncopeltus*, when to my surprise one individual was found (later two others) in which a slight but evident inequality was obvious in a large percentage of the cells at all stages. Upon re-examination of the entire series the interesting discovery was made that in every individual cases could be found of both equality and inequality, *the ratio between them varying widely in different individuals*. In the extreme cases this is perfectly apparent to the eye, so that individuals of predominantly equal or unequal type may readily be distinguished even by casual inspection. In other cases one is often in doubt until large numbers of the nuclei have been tabulated. As examples of the extreme types I give below the results of a study of two individuals (nos. 712 and 760) representing the best material as to fixation and staining. In these a comparison of the X- and

Y-chromosomes as to apparent size was made in one hundred nuclei, taken at random, from each of the following five stages of the spermatogenesis: (1) the pre-synaptic leptotene, (2) the synaptic period (synizesis), (3) the post-synaptic spireme, (4) the first spermatocyte-metaphase, (5) the second spermatocyte-metaphase. The best of these stages for the purpose are the maturation-divisions as seen in side-view (because of the elimination of foreshortening) and the pre-synaptic leptotene (because of the clearness with which both sex-chromosomes may be seen at this time), but in neither individual was the requisite number of side-views of the first division available. In the latter case, therefore, both side-views and polar views have been included. The cases are classed as equal (eq.), unequal (uneq.) and doubtful (dbf.), the latter including those in which there was reason to suspect error due to foreshortening or the like.

PRE-SYNAPTIC				SYNAPTIC			POST-SYNAPTIC			FIRST DIVISION			SECOND DIVISION					
eq.		uneq.	dbf.	eq.	uneq.	dbf.	eq.		uneq.	dbf.	eq.		uneq.	dbf.	eq.		uneq.	dbf.
712	83	10	7	80	13	7	65	23	12	55	37	8	83	9	8			
760	5	93	2	7	89	4	5	86	9	2	95	3	6	87	7			

There is no doubt a considerable error in these figures due to foreshortening, since these chromosomes, though often spheroidal, are often slightly elongated (ellipsoidal), and are of course seen in all positions. But after making a large allowance for this, the contrast between the two individuals is manifest at every stage of the spermatogenesis, and nowhere more so than in side-views of the second division. I have made tabulations of several other individuals which give percentages of equality ranging from ninety down to ten; but in most cases the data from intermediate types are less consistent and the probable error is much larger, for obvious reasons.⁵

From these observations I draw the conclusion that in *Onco-peltus* the X- and Y-chromosomes show a certain tendency

⁵ Compare figs. 9, 10, 14, 15, 24, 25 (equal type, no. 711), 18, 19, 36-40 (equal type, no. 712) with 11, 12, 16, 17, 20, 31, 32, 42 to 44 (unequal type, no. 760). See also photos. 7 to 11.

towards inequality in all individuals, so marked in some cases as to characterize a large percentage of the cells, so slight in others that it can not be distinguished by the eye in more than a small percentage. A noteworthy fact remains to be mentioned. Among my few smear-preparations of *Oncopeltus* is one slide, showing great numbers of nuclei at nearly all stages, in which the X-chromosomes are almost always equal in the growth-period and earlier stages but invariably unequal in the prophases. I distrust this evidence somewhat, for it is notorious that variations of size are very readily produced in smears owing to different degrees of flattening. Were this the only explanation, however, we should expect to see the size-differences as great in the earlier as in the later stages. If the result be trustworthy, it is interesting as indicating the existence of some kind of material difference between X and Y that is expressed in a greater enlargement of one of them at the period when both expand somewhat and undergo longitudinal splitting.

II. THE GROWTH-PERIOD

For the direct study of the actual process of synapsis and its relation to the reduction-division, *Oncopeltus* and *Lygaeus* present practical difficulties that I have thus far found insuperable; hence no attempt will be made to describe synapsis in detail. The transformations of the chromatin during the growth-period will nevertheless be considered at some length, partly in order to trace the complete history of the sex-chromosomes, partly because of the interest of many features presented by the autosomes, and I will also describe certain facts observed in other animals that may help to elucidate some of the problems here encountered.

1. *Outline of the stages*

In *Oncopeltus* it is necessary to distinguish not less than twelve well marked stages following the last spermatogonial division, as follows:

a. (Figs. 47 to 49.) The final spermatogonial telophases, in which the anaphase-chromosomes break up into a confused net-

like structure, and for a short time their boundaries can not certainly be distinguished. In the latter part of this stage the X- and Y-chromosomes become clearly recognizable as compact, deeply staining bodies; but in the earlier stages they too seem to be in a diffused condition. This stage, of short duration, corresponds to Davis's 'Stage *a*' in the Orthoptera ('08), and probably may be compared to the 'resting stage' that has been described as following the last diploid division in many other forms.

b. (Figs. 50 to 51.) Post-spermatogonial nuclei of somewhat larger size, in which the chromatin appears in the form of separate, massive bodies, approximately equal in number to the chromosomes of the diploid groups. Two of these, of more even contour and staining more deeply, are now recognizable as the sex-chromosomes. The other masses are more or less irregular in form, often ragged in texture, and stain more lightly.

c. (Figs. 52 to 55.) The lightly staining masses are in this stage transformed into delicate, closely coiled or convoluted threads, while the sex-chromosomes retain their massive form, and are thus rendered very conspicuous. In the latter part of this stage the fine threads are seen uncoiling or unravelling from the massive bodies to form the leptotene-threads of the following stage.

d. (Figs. 56 to 59.) The pre-synaptic leptotene. The autosomes now have the form of long delicate threads, while the sex-chromosomes retain their massive form as 'chromosome-nucleoli.'

e. The synaptic stage or synizesis (figs. 60 to 61). The threads are now much thicker, stain more deeply, and are closely convoluted in a contraction-figure or synizesis. A plasmasome can sometimes be distinguished at this time, but is usually first seen in the ensuing stage.

f. Post-synaptic spireme (pachytene, diplotene, figs. 62 to 65). Separate thick threads are now again spread through the nucleus, approximately of the haploid number, and in the latter part of the period longitudinally divided. The plasmasome is now nearly always present, though rather small.

g. The diffuse or confused stage (figs. 66 to 67). The double segmented spireme disappears from view, giving rise to a rather

coarse, vague, lightly staining net-like structure, in which are suspended the chromosome-nucleoli and the plasmasome, the latter at its maximum size. In this stage the nuclei remain throughout the greater part of the growth-period.

h. Early prophases (figs. 105, 107). The staining capacity of the chromatin increases, while more definite and apparently *single* threads are evident. The sex-chromosomes are more elongate and longitudinally split. The plasmasome now diminishes in size and disappears.

i. Middle prophases (figs. 108 to 114). The threads rapidly condense, stain more deeply, and draw together to form tetrad-rods, double crosses, double V's, or (rarely) double rings. The sex-chromosomes are short rods, longitudinally split.

j. Late prophases. In these all the chromosomes are converted into compact, deeply staining dumb-bell shaped bodies, rarely quadripartite in outline, which are ready to enter the spindle. This stage is often found in the same cysts with the preceding, all intermediate gradations being readily seen.

k. The division-period, including the two spermatocyte-divisions.

l. Differentiation of the spermatids. Spermiogenesis in the narrower sense.

With various modifications the foregoing stages are found in many Hemiptera, among the best of which for study of the early stages are the pyrrhocorid species *Largus cinctus* and *L. succinctus*. Some doubt exists in regard to Stage *a*; and it is *possible* that in some forms (of which *Largus* may be an example) the spermatogonial chromosomes do not lose their identity at this time but give rise directly to the massive bodies of Stage *b*. In some cases (*Largus*, *Pyrrhocoris*, *Alydus*) the latter part of Stage *g* is characterized by a second synizesis or contraction-figure, in which the autosomes are again closely massed together. In such cases the early prophases are much more difficult to analyze.

I feel confident that the seriation of the stages is correctly determined—indeed the only possible doubt concerns the earliest pre-synaptic stages. The seriation is indicated by the general topography of the testis, which consists of very definite lobes in which

the cysts develop progressively in a nearly continuous series from one end to the other. All the cells in each cyst are nearly, but often not quite, in the same stage. While the order of succession is not demonstrated so accurately as in some objects (e.g., in *Batrachoseps*) it is placed practically beyond doubt by the study of transitional conditions in cysts where slightly earlier and later stages occur side by side.

Throughout the whole complicated series of changes in the autosomes, the sex-chromosomes are at once recognizable at every stage (save the very first) by their condensed and deep-staining character. *Lygaeus* differs from *Oncopeltus* in the fact that the X-chromosome always retains a rod-like form and is longitudinally split at least as early as Stage *f*. In *Oncopeltus* both sex-chromosomes remain in the form of rounded and apparently undivided chromosome-nucleoli up to Stage *h*, when they too assume the form of short, longitudinally split rods. At the period of synizesis the X-chromosome in *Lygaeus* shortens somewhat, but at no time does it assume the rounded form characteristic of *Oncopeltus* and many other forms. In this respect *Lygaeus bicrucis* differs from *L. turcicus*, where the X-chromosome has the form of a much elongated and longitudinally split rod in the early post-synaptic stages, but later contracts to a spheroidal form (Wilson, '05 b). These species of *Lygaeus* remove every doubt, could such longer exist, of the identity of the chromatic 'nucleoli' of the growth-period with a pair of chromosomes.

2. The pre-synaptic period. Stages *a* to *d*

The study of this period in these animals is of much interest in relation to a series of questions, frequently raised in late years, that are of the utmost importance for the theory of synapsis. These are: (1) Are the leptotene-threads of this period chromosomes? (2) Is their number equal to that of the spermatogonial chromosome-groups? (3) Can they be traced directly as individuals to the anaphase-chromosomes of the last spermatogonial division? As will be seen, the facts in the Hemiptera, in the dragon-fly *Anax*, and in certain Orthoptera give good reason to

answer the first two of these questions in the affirmative, while the third remains unanswered.

Stage b. It will be advantageous to consider this important stage before that which precedes it, as there are doubts concerning the latter. This stage and the following one are characteristic of many Hemiptera and Orthoptera, and is seen also in the dragon-fly; and some of these forms are much better adapted for its critical study than are *Oncopeltus* and *Lygaeus*.⁶

In the latter forms numerous cysts in Stage *b* are seen in the region between the spermatogonial cysts and the synaptic zone, often abutting directly upon the former. For this reason I long supposed this stage to follow immediately upon the last spermatogonial division, i.e., to be the last spermatogonial telophase. Such indeed is *possibly* the case in *Largus*, as already stated; but in some other forms it is certainly separated from the telophase by an intervening net-like stage. In *Oncopeltus* and *Lygaeus* Stage *b* is characterized by rather small spheroidal nuclei in which may be very distinctly seen a group of separate, more or less irregular, massive chromatic bodies, the number of which is approximately, in some cases exactly, equal to the diploid number of chromosomes (figs. 50, 51, 71, 72). In preparations but slightly extracted (after haematoxylin or safranin) all these masses stain alike—deep blue or red. Upon further extraction a very striking contrast appears between two of these bodies and the others, the former retaining their deep color and having a fairly even contour, while the latter become pale and are more or less irregular in shape. As will be shown, the two dark bodies are the X- and Y-chromosomes, which may be traced individually through all the succeeding stages up to the spermatocyte-divisions. In *Oncopeltus* they are spheroidal or ovoidal in shape and nearly equal in size (figs. 50, 51). In *Lygaeus* the X-chromosome is much larger than the Y, and always has the form of a more or less elongate rod, which shows a good deal of variation, being sometimes quite straight, sometimes curved in various ways (figs. 71, 72). In

⁶ In my fourth 'Study' ('09 a) I gave a brief account of this stage in *Pyrrhocoris*, illustrated by photographs, describing it as a 'spermatogonial post-phase,' but did not endeavor to work out the history of the autosomes.

Largus and Pyrrhocoris but one dark body is seen; and this, as I earlier showed in the latter case, is the unpaired X-chromosome.

These massive bodies strongly suggest those to which Övertou ('05, '09) has given the name of 'prochromosomes' in the case of plant cells. Since however they differ from the latter in some important respects I will not here employ this term; and for a similar reason will not designate them by Strasburger's term 'gamosomes' ('05), though they undoubtedly give rise to the chromosomes that enter synapsis.

Even a casual inspection of these nuclei is enough to show that the number of chromatic masses is not far from the spermatogonial number of chromosomes, while here and there a nucleus may be found in which this number may be exactly counted. The enumeration is most readily made in the case of *Largus cinctus* where the spermatogonial number is eleven. In this species, which has eleven spermatogonial chromosomes (photo. 33), nuclei may readily be found in which ten of the paler chromatic masses may be definitely counted. In *L. succinctus* their number is often seen to be about twelve (the spermatogonial number being thirteen). In like manner, the number of the pale masses in *Lygaeus* is sometimes seen undoubtedly to be twelve, in *Oncopeltus* about fourteen, the spermatogonial numbers being respectively fourteen and sixteen, though in neither of these species can the number be exactly determined in many cases. I do not hesitate however to draw the conclusion definitely that in these animals *the full diploid number of separate chromatic masses is present in a stage that shortly follows the last spermatogonial division and precedes the formation of the leptotene-threads*. In the dragon-fly, *Anax junius*, there is a closely corresponding stage, but in this case all of the chromatic masses stain nearly alike, and the X-chromosome can often not be certainly distinguished until a little later.

The stage described above evidently corresponds to one in the Orthoptera (Davis's 'Stage b' in *Dissosteira*, Chortophaga and other grasshoppers) and is clearly shown in some of McClung's slides. In all these forms, however, the chromatic masses stain more deeply than in the Hemiptera, are of elongate form, and are

more or less definitely polarized. In *Anax* and the Hemiptera, on the other hand, they are of more less or rounded or irregular form, and show no definite polarization. This is correlated with a corresponding difference in the form and position of the spermatogonial anaphase-chromosomes. In the Orthoptera the latter are in general rod-shaped, with their long axes parallel to the spindle-axis; in *Anax* and the Hemiptera they are much shorter, often rounded in form, and with their long axes (when distinguishable) lying at right angles to the spindle-axis. The conditions described above are occasionally varied by the appearance of one or two deep-staining bodies in addition to the sex-chromosomes, usually of smaller size (cf. the photographs of *Pyrrhocoris* in my fourth 'Study'). Owing to their inconstancy I am uncertain as to their nature.

Whether all of these chromatic masses are chromosomes is a question that probably can not be directly or certainly determined in the case of *Oncopeltus* and *Lygaeus*. We must rely here upon indirect evidence. But there can be no doubt that two of them are chromosomes, for the two deeply staining bodies of *Lygaeus* and *Oncopeltus* may be traced step by step, with no break of continuity, into the two chromatic 'nucleoli' of the synizysis and all succeeding stages, and thence throughout the growth-period into the X- and Y-chromosomes of the maturation-divisions. Since the paler bodies correspond in number to the spermatogonial number of autosomes, and since they undoubtedly give rise to the leptotene-threads that enter the synaptic stage, it is at least a fair inference that they too are chromosomes, or are destined to become such.

Stage a. As stated above, I long supposed the stage just described to follow immediately after the last spermatogonial division; but it now seems certain that in *Oncopeltus* and *Lygaeus*, as in the Orthoptera (Davis, op. cit.) it is preceded by one which more nearly approaches the condition of a 'resting' nucleus. In this stage only the sex-chromosomes can be clearly identified, and there is reason to conclude that in a still earlier telophase not even these can be distinguished.

In certain cysts that obviously precede those of Stage *b* the nuclei are still smaller, the sex-chromosomes more elongated, while the autosomes form a lightly staining, vague net-like structure in which individual chromosomes can not be distinguished. This stage evidently corresponds to Davis's 'Stage *a*' in the Orthoptera, and is well shown in McClung's preparations. A similar stage has been described by several other students of the Orthoptera, especially by McClung.

It is difficult to represent these nuclei accurately in drawings: but a fairly good idea of them may be obtained from figs. 68 to 70, which are from careful studies. They seem to contain a rather coarse and close network, with thickened and irregular nodes of varying size and number. In both species the sex-chromosomes are more elongated than in Stage *b*, and in *Lygaeus* the X-chromosome often assumes an almost vermiform shape, as is shown in the figures. That these nuclei follow almost immediately upon the last spermatogonial telophase is proved both by their small size and by the transitional stages seen in the same nuclei. This is most clearly seen in *Lygaeus*, where the elongate X-chromosome enables us to identify the early spermatocytes with certainty (these chromosomes do not appear as condensed bodies in the spermatogonial nuclei). In the cyst from which figs. 68 to 70 were drawn both sex-chromosomes are perfectly clear in many of the nuclei, but in many the Y-chromosome can not be found, and in a considerable number of nuclei, which seem to lie entirely within the section, not a trace of either sex-chromosome can be seen (fig. 68). In this particular cyst no spermatogonial divisions are seen; but in other cysts in the same region of the testis, nuclei of exactly the same type as those last mentioned (with neither sex-chromosome in evidence) are seen together with the spermatogonial anaphases. That the latter are the final spermatogonial divisions can not be proved; but in *Lygaeus* the evidence seems nearly decisive that there is a short period following the last division in which the identity of all the chromosomes is lost to view. I believe this to be true also in *Oncopeltus*, though the evidence is less satisfactory. On the other hand, it is possible that in *Largus* the final anaphase-chromosomes give rise directly to the massive bodies of

Stage *b*. It is at any rate certain that the telophase-chromosomes in this form retain their identity much longer than in *Lygaeus*, as is shown by figs. 74 and 75, which are connected by all intermediate stages with anaphase-figures in the same cyst. In a recent paper on *Euschistus*, Montgomery ('11) describes a stage that seems to correspond to my Stage *b*, and identifies the massive bodies with the telophase-chromosomes.⁷ I must confess, however, that neither this account nor my own observations on *Euschistus* convinces me that this is correct. It seems to me that we have as yet no safe demonstration in any animal that the pre-synaptic chromosomes are actually the same individual chromosomes as those of the last diploid division.

I am unable to state in exactly what way the massive bodies of Stage *b* arise, for there is no way of demonstrating the seriation at this time, and the change is probably effected rapidly. Different cysts of Stage *b* vary considerably, the massive bodies being more irregular and less sharply defined in some; but I have not gained any clear idea of the succession.

Stage *c*. We may now consider the most interesting changes that take place during the transition to the leptotene stage, the earlier of which may in some cases be seen in the same cysts with the preceding stage. In *Oncopeltus* and *Lygaeus* the minuteness and delicacy of the structures are such that I was long in doubt as to how the process takes place; but *Largus*, *Anax*, and some of the grasshoppers constitute a series in which the same essential phenomenon is seen on a successively larger scale, and which leaves no doubt as to its nature. In all these forms *the process involves the resolution of the paler massive bodies into closely convoluted or coiled threads, which then uncoil or unravel to form the leptotene-threads of the succeeding stage*. The sex-chromosomes, on the other hand, fail to undergo such a transformation, and retain their massive form, though in some cases (*Largus*) there is some evidence that they too may have an internal thread-like structure.

⁷ Arnold ('08) gives a similar account of a corresponding stage in *Hydrophilus*, and describes the massive bodies as conjugating directly two by two, before giving rise to spireme-threads.

A process of this type was long since described by Janssens ('01) in both the *spermatogonial* prophases and the pre-synaptic nuclei of Triton (figs. 27, 67), where it somewhat resembles the resolution into threads of the 'nucleoli' of the germinal vesicle of the same animal, as earlier described by Carnoy and Lebrun ('98). A process more or less similar was described by the Schreiners ('06, '08) in the post-spermatogonial (pre-synaptic) stages of *Tomopteris*, by Pinney ('08) in the spermatogonial prophases of *Phrynotettix*, and especially by Davis ('08) and more recently by Brunelli ('11) in the pre-synaptic stages of *Chortophaga*, *Tryxalis* and other grasshoppers; Grégoire describes a similar process in plant-cells, first in the somatic cells of the root-tip in *Allium* ('06, p. 330), later in the pre-synaptic sporocyte-nuclei ('07, p. 391). The analogous relations discovered by Bonnevie and other recent observers are referred to beyond.

In *Oncopeltus* as the process begins, the pale chromatic masses become looser in texture and more ragged in contour, and each of them gradually assumes the appearance, though somewhat vaguely, of a closely convoluted thread (figs. 52, 53). In the stages that follow (figs. 54, 55) the coiling becomes looser, so that contorted or spiral threads are clearly evident, and at the same time the massive bodies progressively disappear from view. These stages unmistakably show the nature of the process that is taking place. It is now clear that each of the original compact masses (excepting the sex-chromosomes) has resolved itself into a tightly convoluted thread, which is uncoiling to form a leptotene-thread. The spiral or contorted course of the threads is still very evident when the massive bodies as such have disappeared from view (fig. 55), but is finally lost in the completed leptotene-stage (figs. 56 to 59). In *Lygaeus* the process is closely similar and requires no separate description. Figs. 71 and 72 show two nuclei in Stage *b*, in each of which twelve of the paler masses can be counted (not all shown in the drawing), while the X- and Y-chromosomes are conspicuously seen. Whole cysts full of these nuclei are seen in nearly all of my sections. Figs. 73 *a* and 73 *b* show two early leptotene-nuclei of this species after the unravelling is completed.

These stages have been described in *Oncopeltus* mainly because of the importance of following the sex-chromosomes at this period; but, as already mentioned, they are shown more clearly in *Largus*, spermatogonial telophases of which are shown in figs. 74 and 75, and Stage *c* in figs. 76 to 78. Photos. 26 and 27 show nuclei of this form in Stages *b* and early *c*, the character of which I hope will appear in the reproductions. The threads are here coarser and show a more definitely spiral disposition, especially evident as the uncoiling progresses. This is clearly evident in many nuclei in the negative from which photo. 27 is reproduced. Though these nuclei are still rather small, they afford demonstrative evidence in regard to the main fact. I am further confident that the threads are separate and undivided, and that but one thread is formed from each mass; but the latter conclusion is less certain than the former. In the dragon-fly, *Anax*, the facts are similar, and in some respects still more clearly shown. Stage *b* is shown in fig. 85 (the massive bodies all deeply stained); and in fig. 86 (closely similar to Janssen's fig. 67 of the spermatogonial prophases of *Triton*) are shown three nuclei lying side by side, in which appear three successive stages of the unravelling. The spiral disposition of the threads in this form is sometimes conspicuous, and may be clearly seen because of the tendency of the chromatic masses to assume a peripheral position in the nucleus. Not infrequently are seen nuclei like fig. 87 in which a striking effect is given by the uncoiling threads. In such cases it is very evident that the spirals are single, and the evidence is strong that one thread is forming from each massive body.

These stages may be studied to still greater advantage in the grasshoppers, where they have been accurately described and figured by Davis ('08). This observer describes the post-spermatogonial nuclei (Stage *b*) as containing a series of elongated massive bodies, very definitely polarized, approximately equal in number to the spermatogonial autosomes, and having "approximately the same orientation that the autosomes had during the preceding telophases of the last spermatogonial division" (op. cit., p. 38). In figs. 43 and 44 he represents the unravelling of a single thread

from each of these masses, and concludes that each of the latter thus "becomes converted into a *single* chromatin-thread." A study of McClung's préparations, particularly of Achurum, leads me to a confirmation of this conclusion. Stage *b* is better seen in the slides of Phrynotettix than in Achurum; but as the latter shows the unravelling stages more clearly a figure of Stage *b* from this form is here given (fig. 88). In Achurum the unravelling process is quite unmistakable (figs. 89 to 92, less highly magnified than the other figures of the plate; see also photo. 28). The threads here form closely convoluted knots (much like those figured by Janssens in the spermatogonial prophases of Triton), and a spiral arrangement is seldom seen. In Phrynotettix and Mermiria the process is less evident, but appears to be of the same general nature.

Especially in Largus, Anax and Achurum the definiteness of the pictures and the succession of the stages seen side by side in the same cysts or in adjacent ones, entirely excludes, I think, the possibility that they are a merely accidental appearance due to vacuolization of the massive bodies, corrosion-products or fixation-artifacts. The only question is whether the thread that unravels from each massive body is single, double or longitudinally divided. I am nearly certain that the threads are single and undivided. In this respect my conclusions agree with those of Davis, and differ from those recently announced by Brunelli ('11) in the case of Tryxalis. This author describes a similar unravelling process but believes the threads to consist of two separate longitudinal halves which result from a longitudinal split that is evident already in the preceding telophases, and which separate still more widely as they uncoil. The evidence for both these conclusions seems to me very incomplete, as none of the unravelling stages are shown, and the massive bodies of Stage *b* are assumed to arise directly from the telophase chromosomes without proof of this important point. This assumption may be correct, but it seems more probable that Stage *a* has been overlooked by this observer.

The question here involved is so important that I have endeavored to reach a more certain result by study of the analogous processes seen in the spermatogonial prophases. The first of these

cases, as already mentioned, was described by Janssens in Triton. The chromatin-masses ('bloes') from which the spireme-threads unravel are here of irregular shape, and show no polarization, but are nevertheless believed to be directly traceable to the preceding telophase-chromosomes. The threads are already in evidence in the latter, and sometimes show an irregularly spiral course (Janssens's fig. 80) but in the later stages are irregularly convoluted in a manner very similar, as far as can be judged from the figures, to that seen in the pre-synaptic stages of the grasshoppers. Janssens emphasizes his belief that in general a single thread is formed from each 'block,' though in certain cases the latter are double and give rise to two threads. An essentially similar process is described for the pre-synaptic stages. "La première transformation qu'on observe dans les auxocytes est analogue à celle qui annonce le commencement de la division dans les spermatogonies . . . et consiste en une résolution des bloes de nucléine" ('01, p. 68). An essentially similar phenomenon in the spermatogonial prophase is brilliantly demonstrated in two admirable slides of *Phyrnotettix* by McClung, one stained with iron haematoxylin, the other by Flemming's triple method. In this form the 'chromatin blocks' are elongate and polarized (fig. 93, photos. 29 to 31), and the thread later forms a beautiful and very definite spiral, as was first described by Pinney ('08). This observer describes the spiral threads as formed separately within the vesicles or sacculations to which the preceding anaphase-chromosomes give rise (as first made known by Sutton '00, in *Brachystola* and confirmed by several others subsequently).

As far as can be judged from the figures and brief description of Pinney, the threads are formed as rather loose and open spirals directly out of a fine reticulum within each chromosome-vesicle. The rather limited material at my disposition shows somewhat different conditions, though confirming the main point. The conditions in McClung's slides differ from those described by Pinney in that none of the resting nuclei or early prophase show the nuclear sacculations so distinctly, nor is the chromatin so diffuse—differences which may well be due to the fact that none of the earlier generations of spermatogonia are shown. In these nuclei

the thread-formation is preceded by a stage in which the chromosomes appear in the form of deeply staining, elongated, and more or less definitely polarized bodies (fig. 93, photo. 29), ragged in contour and loose in texture, but showing as yet no definite coiled thread. This condition must shortly precede the thread-formation because the latter may clearly be seen in other nuclei in the same cysts. Whether these bodies are individually derived from the anaphase-chromosomes of the preceding anaphase can not here be determined, but Miss Pinney's observations make it highly probable that such is the case. In any case, already in the early prophases each of these masses is seen to be resolving itself into a closely coiled or convoluted thread, similar to that seen in the pre-synaptic stages but disposed in more definitely spiral fashion (figs. 94 to 96, photos. 30 to 32). In some cases there are indications that each of these spirals is still enclosed in a more or less separate nuclear sacculation, in other cases this can not be seen. In some cysts the threads are seen tightly coiled within the massive bodies at one side of the cyst, while stages of uncoiling are seen progressively towards the opposite side. In slightly later stages all gradations are seen in the uncoiling of the threads to form *separate* threads, which still show a distinct spiral course even after they have begun to shorten and thicken (fig. 96, photo. 32). From this stage it is easy to trace every step up to the time when the prophase-chromosomes are about to enter the metaphase. The longitudinal division is not evident until the uncoiling is well advanced, and the two halves remain in close apposition until the metaphase.

The points that I would here emphasize are:

1. The extreme clearness with which the spiral threads are seen, which removes every possible doubt as to what is taking place.
2. The fact that the threads are separate from the time of their first formation. Apparently there can be no question here of a continuous spireme.
3. The transitional conditions seen in the same cysts, which prove these stages to be prophases, not telophases. In this re-

spect the phenomenon is different from that discovered by Bonnevie ('08, '11) in *Ascaris* and *Allium*, where the spiral thread is formed in the telophase-chromosomes and uncoils to form the thread-work of the resting nucleus.

4. The certainty that the spirals are single, not double, i.e., do not consist of two interlacing spirals, as has recently been described in the telophase-chromosomes of *Tryxalis* by Brunelli ('10), and in the final anaphase-chromosomes of *Amphibia* by Schneider ('11) and Dehorne ('11).

5. The strong evidence thus afforded that only one thread arises from each chromatin-mass.

There can be no doubt that the process here so clearly demonstrated is of the same general nature as that seen in the pre-synaptic nuclei of these animals and of the Hemiptera. I therefore consider it at least probable that in the latter case also a single thread is formed from each chromatin-mass and hence that the number of pre-synaptic leptotene-threads is equal to the diploid number of chromosomes.

Résumé of Stages a to c. The close parallel that exists between the pre-synaptic stages of the Hemiptera, Odonatata and Orthoptera is obvious. In all these forms the pre-synaptic chromosomes first appear in the form of massive 'prochromosome'-like bodies, approximately of the diploid number, of which one (*X*) or two (*X* and *Y*) are already recognizable as the sex-chromosomes by their more compact structure, regular contour, and deep-staining quality. Each autosome is converted into a tightly coiled or convoluted thread which ultimately unravels to form a leptotene-thread of the stage which immediately precedes synapsis. This process is clearly analogous to that seen in the spermatogonial prophases, and in each case the evidence is that a single thread arises from each massive body. The pre-synaptic leptotene-threads are thus seen to be of the same nature, and probably of the same number, as the spermatogonial prophase-threads, and are therefore to be regarded as forming a diploid group of chromosomes. The sex-chromosomes, on the other hand, persist in the massive form to constitute 'chromosome-nucleoli.'

which may be traced into and through the growth-periods.⁵ It is however very doubtful whether the massive bodies of Stage *b* can actually be traced individually back to the anaphase-chromosomes of the preceding division, though this *may* be possible in some forms.

Stage d. The leptotene-nuclei. It is impossible to draw any definite line of demarkation between this stage and the preceding one, since they are connected by insensible gradations. An excellent idea of these stages is given by Miss Hedge's careful drawings (figs. 56 to 59; cf. photos. 7, 8). In the earlier nuclei the threads still have a more or less spiral or wavy course, and still show distinct evidence of clumping together in masses. A little later both these appearances are lost, and the threads form an evenly diffused, delicate spireme, always separated from the nuclear wall by a considerable clear space. Still later the threads become somewhat thicker, more open in arrangement, and stain a little more deeply (figs. 58, 59, 73 *a*, 73 *b*, 78 to 80).

These nuclei are now ready to enter the synaptic or synizesis stage, which immediately follows. They show essentially the same characters in *Oncopeltus*, *Lygaeus*, *Largus*, and many other Hemiptera; but their composition is difficult to analyze precisely. It is certain that the spireme is not continuous at this stage, for free ends of the threads are readily seen; but the number of threads can not be determined. In general they show no trace of polarization, though in *Lygaeus* traces of such an arrangement are sometimes visible. Whether the threads branch or not is a very difficult question. At first sight they give the impression that they do branch; and in my fourth 'Study' (on *Pyrrhocoris*) I described them in fact as forming a "net-like structure in which traces of a spireme-like arrangement may sometimes be seen." The more carefully one studies these nuclei, however, the more doubtful this becomes. Certainly the threads may often be followed

⁵ The mitotic transformation of the massive bodies is not however diagnostic of the autosomes, for in some of the Orthoptera, as McClung and his successors have shown, the X-chromosome is also converted into a closely convoluted thread at a later period. I have some reason to suspect that the X-chromosome of *Largus* may also consist of a very tightly convoluted thread in the earlier stages; but there is never any sign of its uncoiling, and in the later stages it appears homogeneous.

individually for a considerable distance without branching; and it is my belief, after prolonged study, that the threads do not really branch or form a network, though such an appearance is often given by fine strands of 'linin' (perhaps coagulated nuclear sap) connected with the threads.

A point to be emphasized is that these threads do not show the least sign of longitudinal division, and in this respect offer a marked contrast to the longitudinally double threads seen in the post-synaptic stages.

As Stage *c* passes into Stage *d*, the contrast between the sex-chromosomes and the others becomes still more pronounced. In *Oncopeltus* the former often become nearly spheroidal in shape, and stain so intensely as to appear exactly like chromatic nucleoli. In *Lygaeus* they stain with equal intensity, but still retain more or less of a rod-like form, particularly in case of the X-chromosome. In *Largus* (as in *Pyrhocoris*) the unpaired X-chromosome becomes as a rule spheroidal. In all these forms the sex-chromosomes always occupy a peripheral position with respect to the mass of chromatin-threads, sometimes in the clear space outside the latter, more often embedded in its peripheral zone. A very striking fact (to which I formerly called attention in case of *Pyrhocoris*) is that the sex-chromosomes in these stages are always separated from the threads by a vacuole-like space. This is most conspicuous in *Largus* (figs. 78 to 80) where the vacuole is unusually large and clearly defined; but it also appears in the other forms when seen in the right position. No definite wall to the vacuole can be seen, but the chromatin-threads are often seen encircling its outer limit, as if lying upon a definite substratum. This fact is interesting as indicating that the sex-chromosomes really lie in separate compartments or chambers of the nucleus, even though their walls can not be seen. Is this, conceivably, true of other chromosomes, and may this possibly be the basis of the genetic continuity of chromosomes in general?

3. Stage e. The synaptic period. Synizesis

We now approach a problem that I have thus far found insoluble in these animals, and which will therefore be considered very briefly. This involves the changes by which the leptotene-nuclei pass into the pachytene stage, which here begins with the contraction-figure, or synizesis. This stage is initiated by a rapid thickening of the threads, accompanied by an increase in staining capacity and a further contraction of the mass which they form. A very good idea of this stage may be obtained from fig. 60, which is carefully studied in every detail. As this figure shows, the synaptic knot distinctly shows two kinds of threads, thick and thin, closely convoluted, but showing no definite polarization or other visible arrangement in loops. The results shows that the process of synapsis must be in progress at this time, but the closest study has thus far failed to reveal the true relation of the thick threads to the thin, and I doubt the practicability of determining precisely what is taking place. In these Hemiptera, as Digby has recently remarked of *Galtonia*, "synapsis faces one as an impenetrable wall" ('10, p. 739). A little later the synaptic knot undergoes still further contraction (fig. 61) and is till more difficult to analyze; but in favorable cases it may be seen to consist of thick threads, closely convoluted, and still showing no trace of polarization. This stage evidently corresponds to the early pachytene of other forms; but the 'bouquet' figure, so characteristic of many animals, seems to be entirely wanting here, and I have found no indication of it in any of the Hemiptera.

Of one hundred nuclei of this stage in *Oncopeltus*, taken at random, seventy-five showed X and Y entirely separate, sometimes on opposite sides of the synaptic knot, while in twenty-five cases they lay side by side, just in contact. Not one of these nuclei has been found after a search of many hundreds, in which these chromosomes were fused, or even flattened together. In *Lygaeus*, on the other hand, there is a stronger tendency for these chromosomes to come together at this time, one hundred nuclei showing them separate in forty-five cases and in contact in fifty-five. In the latter case they are often pressed together to form

an unsymmetrical dumb-bell shaped body (photo. 12) but are never fused to form a single body. In thirty-six of the foregoing fifty-five cases in *Lygaeus*, *X* and *Y* were attached end to end (photos. 12, 13), in seventeen side by side (photo. 13)

There is a good deal of variation in the degree of contraction, even in cells of the same cyst; and this may be due in part to differences of response to the fixing agent. That the contraction figure can not be regarded as an artifact, however, is proved by the fact (which I briefly described in my fourth 'Study') that in some Hemiptera it may be readily seen in the living cells, as has also been shown by other observers. Gates ('08) has suggested, in case of certain plants, that the synizesis is not produced by a contraction of the chromatin-mass but by enlargement of the nucleus due to rapid accumulation of liquid about the chromatin. Such a view can hardly apply to these insects, I think, though studies of the living material would give a more trustworthy result than those upon sections.

The synaptic knot often lies excentrically in the clear space. Just outside it, or embedded in its periphery lie the sex-chromosomes, still surrounded in many cases by the vacuole, though this is now less evident. They retain the same appearance as in the preceding stage, except that in *Lygaeus* they are somewhat shorter than before. In none of these Hemiptera does either sex-chromosome elongate, or show any definite relation to the nuclear pole at this stage. In this respect these animals differ markedly from some of the Orthoptera, where the *X*-chromosome becomes elongated and takes part in the general polarization of the chromatin in the 'bouquet' stage. There is no evidence of a giving off of material from this chromosome or from the nucleus at this time.⁹

4. Stages *f* and *g*

Stage f. The post-synaptic spireme. Pachytene and diplotene.
In the stage immediately following synizesis the chromatin-threads quickly spread apart through the nuclear cavity, and are

⁹ Cf. Moore and Robinson ('04) and Morse ('09) on the cockroach, Buchner ('09) on *Gryllus*.

now very clearly seen to be separate, constituting a segmented spireme. All the threads still stain deeply and are very much thicker than in the leptotene-stage; hence these nuclei may be called the pachytene-nuclei. In the earlier part of this stage it is uncertain whether the threads are longitudinally split or not; in many cases the closest study fails to reveal such a split in sections (figs. 62, 63), though in smears (fig. 65, photo. 10) the split is very clearly seen in nuclei that seem to belong to this period. In the later part of this period the threads become still thicker and shorter and very often show a conspicuous longitudinal cleft. This is less readily seen in *Oncopeltus* and *Lygaeus* (figs. 64, 83, 84) in which, indeed, the threads sometimes do not show a trace of such a cleft at this time (which I attribute to defective fixation). In *Largus*, on the other hand, the cleft appears in the most conspicuous way, especially in sections fixed with Hermann's fluid (figs. 81, 82), where the threads are often seen to consist of double rows of granules often showing a distinctly paired arrangement.

In *Oncopeltus* and *Largus* the sex-chromosomes are at this time hardly changed, still having the form of undivided, rounded chromosome-nucleoli. In *Lygaeus*, the Y-chromosome is still of this type, but the X-chromosome (usually near the nuclear membrane) is now very clearly split lengthwise (fig. 84), in which condition it persists from this time throughout the whole growth-period. The plasmasome is considerably larger than before although not yet at its maximum size.

The number of chromosomes (separate chromatin-masses) is now obviously approximately half that of the diploid groups. In *Oncopeltus* and *Lygaeus* this can be determined only approximately; but it is certain that the number is not far from the reduced or haploid number—that is to say, there are in *Oncopeltus*, in addition to the two chromosome-nucleoli, about seven separate diplotene-threads, in *Lygaeus* about six. In *Largus cinctus* (where the spermatogonial number is eleven) nuclei may readily be found in which the number of double threads may be exactly counted. Such nuclei show, in addition to the single chromosome-nucleolus, five double threads (figs. 81, 82) of which

one is much longer than any of the others and evidently corresponds to the large pair of chromosomes that are a constant feature of the diploid groups in this species (photos. 33, 34). From these facts it is clear that each of the double threads is a bivalent, which corresponds to a pair of spermatogonial chromosomes, and that *synapsis must have taken place during the period of contraction or synizesis*, as many other observers have concluded in both animals and plants.¹⁹ In what manner synapsis takes place, and whether the longitudinal halves of the diplotene-threads represent the original conjugants in side by side union are questions that here present very great practical difficulties to direct observation.

Stage g. The diffuse or confused period. The diplotene-nuclei now undergo a remarkable transformation, characteristic of many Hemiptera, in the course of which the double threads as such completely disappear from view, giving rise to a diffuse, lightly staining net-like stage in which the boundaries of the individual bivalents are indistinguishable (figs. 66, 67, 97, photos. 11, 14, 16). In *Oncopeltus* and *Lygaeus* I have found it impossible to arrive at any clear notion as to the exact nature of this transformation. In Hermann preparations of *Largus* all the transitional stages are shown with great apparent clearness, yet even here it is difficult to reach a certain result. This question—one of the most important involved in the maturation-process—will, I believe, repay careful study in smear-preparations, which I hope to undertake hereafter with more adequate material than I have at present.

As the process begins, the threads become less regular and at the same time longer and thinner, while the longitudinal cleft is still more evident. A little later the two halves of the double threads become more or less contorted, more granular and irregular in structure, and at the same time are often seen to be separating in an irregular way (figs. 101–103). By the continuation of this change the double threads as such disappear from view, and the whole nucleus is traversed by rather thin, irregular, con-

¹⁹ In *Syromastes* Gross ('04) believed that the somatic or diploid number of double threads could be counted in the post-synizesis stages, and that synapsis took place at a later period.

torted, more or less interrupted granular threads, which often seem to branch more or less. These nuclei show a certain resemblance to the pre-synaptic leptotene-nuclei of Stage *d*; but both their position in the testis and their structure render a confusion between these stages impossible. When the process is completed the threads are greatly diminished in staining-capacity, seem to branch more freely, and in *Oncopeltus* and *Lygaeus* often give almost the appearance of a network with thickened nodes (figs. 66, 67, 97). In *Largus*, however, the threads remain more in evidence, and the nuclei do not so nearly approach the 'resting' condition (fig. 104).

At the height of this stage it is, I believe, quite impossible to *distinguish* the individual chromosomes (bivalents) or to analyze exactly the composition of the nuclei. I nevertheless incline to the conclusion that the autosomes do not actually lose their identity at this time. The phenomena which follow in Stage *h*, especially as shown in *Protenor*, give considerable reason to conclude that the prophase-figures are already formed in the diffuse stage but are lost to view by their intricate extension, contortion and interlacing. In *Euschistus*, as recently described by Montgomery ('11), the confused period is much less marked; and this observer believes that the bivalents may be individually recognized at every period. In *Tomopteris* and *Batrachoseps* the confused period is entirely omitted.

In the condition described the nuclei remain throughout the greater part of the growth-period. In *Oncopeltus* the sex-chromosomes remain always spheroidal or ovoidal (photos. 11, 16) and apparently undivided. In *Lygaeus* both sex-chromosomes (of which a more detailed account is given at p. 384) are rod-like and longitudinally split (photos. 13 to 15). In *Largus* the X-chromosome is spheroidal but often shows a small but very distinct central cavity. In all these forms the plasmasome is conspicuous throughout, and attains its greatest size in this stage. In *Oncopeltus* and *Lygaeus* the chromatin undergoes no contraction during this period. In *Largus*, on the other hand (as in *Pyrrhocoris*, *Alydus* and some others) the latter part of this period is characterized by a very marked second contraction-figure or synizesis,

forming a spheroidal and rather dense mass separated from the nuclear wall by a considerable clear space (cf. Gross's figures of this stage in *Pyrrhocoris*, '07).

5. *Stages h to j. The prophases*

a. *The bivalents.* At the end of the diffuse period the nuclei undergo a rapid change which marks the appearance of the definitive prophase-chromosomes. This is accompanied by a progressive condensation and increase of staining capacity, which reaches a climax in the final prophases, and by the disappearance of the plasmasome. These changes may be studied to better advantage in smears than in sections, and is better shown in my material of *Protenor* than in the other forms. As seen in sections, the initial stage (figs. 105, 106) shows the nuclear threads more distinct, less crowded and straighter, often giving an appearance somewhat similar to the beginning of Stage *g*, but the bivalents are not yet defined. In smears of *Protenor* (figs. 115 to 117) it is clearly apparent that the threads are separate, *single* (i.e., not longitudinally split) and much contorted. A little later the threads are seen to be forming themselves into the characteristic bivalent figures, still in a very diffuse and irregular form, but plainly showing their individual boundaries, and in some cases also their characteristic forms (figs. 107, 108, 118, 119, photo. 17). In *Protenor* the *m*-chromosomes are first clearly seen at this time but are much less definite in contour than in the following stage. As the condensation proceeds the bivalents become more definite in shape and can be more readily analyzed. In Stage *i* (figs. 109-14, photos. 18 to 23) they have the forms which have been familiar to us since the early work of Paulmier ('98, '99) on the Hemiptera. The most characteristic of these is (1) the double cross, consisting of four arms, at right angles to each other, and longitudinally split. The four arms may be equal in length. More commonly one pair is shorter than the other. In the later stages the four arms typically lie in the same plane. In earlier ones they are often curved; and the two longer arms may be curved towards each other until they nearly meet to form a ring. (2)

In some cases the two arms actually meet, uniting to form a closed ring, of the type first made known by Paulmier ('98) and often observed since, both in insects and in other animals; such as *Tomopteris*, or the grasshoppers; but this type is much rarer in *Oncopeltus* and *Lygaeus* than in some other species. (3) The third type is that of the tetrad-rod, which consists of a straight rod, which shows both a longitudinal split and a transverse median suture. These forms are readily deducible from the double cross by reduction and final disappearance of the lateral arms, the position of which is now indicated by the transverse suture. As will be shown hereafter (especially in the case of *Protenor*) the double crosses undergo in their later stages precisely this change; but the evidence indicates that some of the tetrad-rods never pass through the double cross stage. (4) The fourth type is the double-V, best described as a V-shaped figure that is longitudinally split in the plane of the two branches, from the apex of the V towards the free ends, accompanied by a greater or less degree of separation of the two halves thus produced. Figures of this type are especially common in the earlier stages (fig. 108, photos. 17, 18) and may be recognized soon after the beginning of Stage *h* in a much more elongate form, as shown in fig. 107, photos. 17 (from a smear-preparation). In the final prophase (Stage *j*) all the bivalents finally condense to form dumb-bell figures, though the double crosses (now much condensed, and often more or less opened out in a ring form) may sometimes still be distinguished in the early metaphases. In the course of this process the lateral arms of the crosses sooner or later disappear, and a cross constriction appears at the points where they have been. These conditions will be more fully considered later in the case of *Protenor*.

Owing to the uncertainty regarding synapsis and the impossibility of tracing the bivalents individually through the confused period, it is not possible to offer more than a somewhat conjectural interpretation of the origin and relationships one to another of these various forms. Paulmier, McClung and other earlier students of the insects assumed the primary type to be a tetrad-rod, representing two univalent chromosomes, united end to end, and longitudinally split. From this type the double cross was assumed

to arise by a drawing out of the central region of each longitudinal half from the synaptic point to form the 'lateral arms' of the cross. The ring-form was supposed to arise by a secondary bending around of the two principal arms until the free ends united; the V-forms by a sharp flexure of the bivalent at the synaptic point (cf. Paulmier, '98, '99). On the whole, however, it seems to me that the evidence points more strongly to the opposite interpretation, first clearly worked out by the Schreiners ('06) for the closely similar figures seen in *Tomopteris*. According to this, the original condition is that of two parallel threads or rods, in parasynaptic association, each of which sooner or later undergoes longitudinal fission. The rings are described as arising by an opening apart of the two rods along their middle portions while remaining attached by both ends; the V's by an opening apart from one end, while remaining attached at the other; and from the latter, by complete opening out of the two limbs until they are in a straight line, arise the tetrad-rods. From the latter the crosses are readily derived by drawing out of the lateral arms in the manner assumed by Paulmier.

Though all this is somewhat hypothetical as applied to these insects, I consider it the more probable view for several reasons. The first of these is the evidence that the lateral halves of the diplotene-threads begin to separate already at the end of Stage *f* as the nuclei are passing into the confused stage, and the correlated fact that in the initial prophase the bivalents are seen drawing together out of more or less widely separated *single* threads. A second is the prevalence of the double V-figures in the early prophase, and their gradual disappearance as the prophase advances. It is evident that these V-figures are opening apart in these stages, not closing up. Elongated V's with their limbs often nearly parallel (figs. 107, 108, photo. 17) are commonly seen in smear-preparations of these stages, and in slightly later ones all intermediate stages connect them with the tetrad-rods or double crosses (figs. 109 to 114, photo. 18). These facts are quite independent of any particular conception of synapsis, but they seem to fit best with the view that the original type is a longitudinally double rod following parasynapsis, as maintained by the Schrei-

ners. This view receives strong support from Montgomery's recent paper on *Euschistus*, in which all stages of such opening out are shown, and in which the process of parasynaptic union is described in detail. It may be pointed out that the accurate figures of Sutton ('02) from smear-preparations of *Brachystola*, are entirely in accordance with such an interpretation, as has been also indicated by Grégoire ('10). I nevertheless adopt this conclusion only in a provisional way, as it is still based to large extent upon indirect evidence much of which is not inconsistent with the earlier and opposite conception of Paulmier.

The facts that have been described, especially as seen in *Protenor*, point very definitely to the conclusion that the initial stages of the formation of the bivalents are passed through with as the nuclei pass into the confused stage, and that they do not really lose their identity in this stage but are only lost to view by their looseness of structure, great extension, and intricate entanglement. This interesting question will repay more adequate study; for if my conclusion be correct it may help us to solve the difficult problem of the disentanglement of the leptotene-loops in the synaptic process of such forms as *Tomopteris* and *Batrachoseps* (cf. p. 407).

b. *The sex-chromosomes.* The history of the sex-chromosomes during these stages is very easily followed throughout, particularly in smear-preparations, and affords a complete demonstration of their identity with the chromatic 'nucleoli' of the growth-period. In Stage *h* these chromosomes almost always lie close against the nuclear wall, and in *Lygaeus* still show but little change; both retaining the form of short longitudinally split rods. In *Oncopeltus* they show a marked change, being now more or less elongated into a rod-like form, often a little irregular in shape, and now for the first time plainly *longitudinally split* (figs. 105, 106). In Stage *i* they are regular, short, compact longitudinally divided rods, essentially like those of *Lygaeus* save for their nearly equal size. This may be studied to best advantage in smear-preparations, where the composition of the chromosome-groups may be completely analyzed. In such preparations the total number of chromosomes in *Oncopeltus* is nine, including seven bivalents

and the two univalent sex-chromosomes. The latter may at once be recognized by (1) their smaller size, (2) more compact texture, and (3) simple, rod-like form and longitudinal split (figs. 108 to 110, photos. 20 to 23). In both sections and smears all gradations are seen between these nuclei and those of the growth-period which remove all doubt as to the identity of these chromosomes with the chromatic 'nucleoli' of the latter. On the other hand, it is easy to trace these chromosomes step by step through the later prophases into the two small chromosomes of the first division. In these stages the double rods are seen progressively shortening until they assume the dumb-bell shape in which they enter the spindle (figs. 112-114). It is clear from the transitional stages that the transverse constriction of the dumb-bell corresponds to the original longitudinal split of the rod before its shortening, while the long axis of the dumb-bell represents the original transverse axis of the rod. The apparent 'transverse' division of the dumb-bell is therefore in reality a longitudinal division.

We may with advantage consider at this point some very interesting features presented by the X-chromosome in *Lygacus bierucis*¹¹ especially during the stages preceding the prophases. In the earliest stage (*a*) this chromosome is an elongate, almost vermiform body, which appears homogeneous in structure (figs. 69, 70). In Stages *b* to *d* (figs. 71 to 73) it is shorter and thicker, and still usually appears homogeneous, though in much extracted preparations of Stage *c* it may appear longitudinally divided. In Stage *e* (synizesis) it is considerably shorter and shows no sign of division (photo. 12). In Stage *f* it is again more elongated and unmistakably split lengthwise (photos. 13 to 15), and in this condition persists throughout the whole growth-period, gradually shortening in the prophases until it assumes a dumb-bell shape, quite as in *Oncopeltus* (photo. 25).

At every period from the post-synaptic spireme onwards many cases may be found in which the double rod appears nearly or quite homogeneous (figs. 84, 98, 99, 100 *a, b*; photos. 14, 15); but

¹¹ This account applies only to this species. The facts in *L. turcicus* are very different, as already mentioned (see Wilson, '05 b, '06).

in many other cases it very clearly shows a double series of varicosities that are accurately paired in the two longitudinal halves, as if the rod originally consisted of a series of large granules or segments that afterwards underwent longitudinal fission. Some of the various forms that appear are represented in fig. 100. Of these forms one is far more frequent than any of the others—, that, which shows three pairs of segments (100 *d*), which in the best cases are very sharply marked, in others less distinct though evident, in others barely perceptible. In some cases (usually more elongate forms), four segments are apparent (fig. 100, *c*), but no case has been seen with more than four. In a few cases, where the rod seems to be shorter, less than three segments appear, and an almost quadripartite form results (fig. 100 *e*). Some of these cases are obviously due to a sharp curvature of the rod, so that in foreshortened view only the end segments are seen; but I have seen a few cases in which the rod seems to have simply shortened and two pairs of the segments seem to have fused together. In fig. 100 *f* the rod seems to show six segments (the only such case seen); but it is nearly certain that this represents the *X*- and *Y*-chromosomes lying end to end, as a separate *Y*-chromosome can not be found elsewhere in the nucleus. This case, as well as others where *Y* is separate, indicates that the *Y*-chromosome also may consist of segments, but not more than two such have been seen in any case. Figs. 100, *g* and *h*, show two isolated *Y*-chromosomes of the homogeneous type.

It seems to me hardly possible that this striking appearance is an accidental artifact, first because of the frequency of the tri-segmental type, and second because of the correspondence of the segments of the two halves in each double rod, which is often rendered more striking by a decided inequality of the segments (well shown in fig. 100 *f*) in each half. All such cases that I have seen show the segments accurately paired. For these reasons I believe the segmented structure to be comparable to the linear arrangement of 'chromatin-granules' so often described in the spireme-threads of the ordinary chromosomes, and to be an expression of some kind of internal structure in the *X*-chromosomes. These facts may be added to the evidence reviewed in my preced-

ing 'Study' ('11 a) that the X-chromosome (like other chromosomes) is a compound body. They help us to understand how an X-chromosome that is originally single may break up into two or more components that behave as separate chromosomes in the diploid groups but become associated in a coherent group ('X-element') at the maturation-period (Payne, '09, Wilson, '11, Edwards, '10), and provide a still more definite basis for the conclusion that this chromosome may be the bearer of many other factors than the one for sex (Wilson, '11, Morgan, '11, Gulick, '11). The bearing of this on sex-limited heredity is obvious.

It is a very important fact that *at no time in their history do the individual sex-chromosomes in these Hemiptera exhibit a cross-form or tetrad structure comparable to that which is so characteristic of the bivalents*. Such a tetrad structure only appears when the two sex-chromosomes are united to form a bivalent— as is seen for instance in *Brochymena* or *Nezara* (Wilson, '05 b, '11 a). The only apparent exception to this is the X-chromosome in *Lygaeus*, as already mentioned; but this exception is evidently only apparent. The essentially bipartite structure of these chromosomes is a significant fact that is obviously correlated with their univalent nature, and with their approaching *single* division in the course of the two spermatocyte-divisions. The wider implications of this will be considered in Part III, in connection with the facts seen in *Protenor*.

6. Comparative considerations regarding the maturation-period

A comparison of the growth-period in these Hemiptera with the conditions seen in such forms as *Tomopteris* or *Batrachoseps* shows some striking, though I think secondary points of difference.

In the first place, the formation of compact, massive bodies from which the leptotene-threads unravel in the pre-synaptic period, which is so characteristic of these insects, seems not to take place in *Batrachoseps* and some other forms; though, as will be indicated beyond, the Schreiners have found indications of an analogous process in *Tomopteris*.

Secondly, the 'polarized amphitene, or 'bouquet-stage,' that is characteristic of *Tomopteris*, *Batrachoseps* and other forms, seems

to be entirely wanting in these insects, where in its place we find the closely convoluted and apparently non-polarized synaptic knot or synizesis. The controversy as to whether the latter is an artifact, due to the coagulating effect of the reagents, seems to be terminated by the fact, determined by Sargant ('97), Overton ('05), Berghs ('04), Oettinger ('09), and myself ('09 a, '09 b) that the synizesis may be clearly seen in the fresh (living?) material immediately after gentle teasing apart of the cells in a normal fluid (Ringer's solution) in which the spermatozoa continue actively to swim. Neither at this stage nor in those that immediately precede or follow is there the least sign in these animals of an elongation of the sex-chromosomes or of a giving off of nuclear material to the protoplasm.

Third, in *Tomopteris* and *Batrachoseps* the pachytene-loops formed in synapsis persist as such throughout a large part of the growth-period, without undergoing at any period an apparent loss of identity in a 'diffuse' stage such as is so characteristic of the Hemiptera. In *Batrachoseps* the pachytene-loops become longitudinally divided ('diplotene') near the end of the growth-period, when they give rise directly to the prophase-figures. In *Tomopteris* the diplotene-threads are apparent at a much earlier period (Schreiner), but here too give rise directly to the prophase-figures. In the Hemiptera here considered the diplotene is likewise formed very early, but the diffuse stage is interpolated between it and the definitive formation of the prophase-figures, and the greater part of the growth-period is passed in this condition (in some cases accompanied by a second contraction-figure in the later period). There is, however, an analogy in this respect between these Hemiptera and *Tomopteris*, where the Schreiner describe and figure ('06, p. 19, figs. 31, 32) a stage following the early diplotene in which the parallel halves of the double thread become longer, thinner, less regular, and spread more or less widely apart, though still retaining their connection at certain points. It is very probable that this process corresponds to that which marks the beginning of the diffuse stage in the Hemiptera, but does not proceed so far; and that in this respect *Tomopteris* is intermediate between these animals and the Amphibia. Perhaps

we may here find a clue to the more extreme forms of diffusion observed in the oogenesis of many animals and in the ordinary somatic nuclei.¹²

As regards the problem of synapsis and reduction, the existence of the synzesis and diffuse stages renders the Hemiptera very unfavorable objects as compared with *Tomopteris* or *Batrachoseps*, and we are here thrown back upon analogies. Emphasis may however be laid upon the essential similarity of the prophase-figures in *Tomopteris* and these insects; and if my interpretation of the diffuse stage be correct, it is probable that these figures have essentially the same mode of origin from the diplotene-threads. Following this analogy, I provisionally assume the latter to follow an original side by side union, or parasynapsis not an end to end union or telosynapsis, as was assumed by Paulmier, Montgomery and (in Orthoptera) McClung, Sutton, and more recently by Davis. In his latest paper ('11) Montgomery rejects his former interpretation in favor of the one here adopted. If the double cross-figures (or the tetrad-rods) arise in the manner assumed, it is clear that their 'transverse' division is the last remnant of the original longitudinal cleft of the diplotene-thread; and it is certain, as Paulmier first showed, that this 'transverse' division corresponds to the plane of the first spermatocyte-division. If we accept this, and if the original longitudinal cleft of the diplotene corresponds to the plane of synapsis, it follows that the first spermatocyte-division is the 'reduction-division,' as Paulmier and Montgomery concluded. I repeat, however, that this conclusion is here adopted only in a tentative way; since the case is by no means proved; and, as will appear, my conception of the reduction-division differs materially from the one more commonly held.

As regards the sex-chromosomes, on the other hand, all is clear. The observations here recorded remove every doubt, I think, in

¹² A more or less wide divergence of the longitudinal halves of the diplotene-threads appears to be the rule among many animals and plants. It has been especially emphasized by Grégoire ('04, '10) who has called attention to the striking contrast in this respect between the bivalent chromosomes of the maturation-period and the longitudinally split spireme-threads of the somatic divisions. See also Strasburger, '09, pp. 98 to 100.

regard to the following points. First, it is certain that *each of these chromosomes divides but once in the course of the maturation-process, namely, in the first division; and this division is clearly longitudinal and equational. The second 'division' of the XY-pair is obviously not a division at all but only the disjunction of two separate chromosomes that have for a short time been in contact without loss of their identity.* This process is an evident and typical reduction-division in the original sense. In these animals, therefore, it is quite certain that the XY-pair undergoes a process of 'post-reduction' (cf. Wilson, '05 c). It is a remarkable fact, proved by the studies of Stevens, that in the Coleoptera and Diptera the XY-pair follows the reverse order, as is also the case with the *m*-chromosomes of the coreid Hemiptera.¹³

7. *Comment on the sex-chromosomes in Oncopeltus*

The extremely close correspondence between *Oncopeltus* and *Lygaeus* at every stage of the spermatogenesis leaves not the least doubt of the identity of the sex-chromosomes of the two forms. Apart from the size-differences of these chromosomes, *Lygaeus* differs from *Oncopeltus* only in (1) the retention throughout of a rod-like form by the X-chromosome, (2) the earlier appearance of the longitudinal split in both sex-chromosomes, (3) a slightly more marked tendency for the sex-chromosomes to conjugate at the time of general synapsis. On the other hand, the sex-chromosomes of the two forms agree in all the characteristic peculiarities of these chromosomes shown in the Hemiptera generally, namely, (1) the retention of a compact and deeply staining character from an early pre synaptic period down to the spermatocyte-prophases, (2) their division as separate univalents in the first spermatocyte-division, (3) their subsequent conjugation to form a bivalent, which occupies a nearly central position in the second spermatocyte metaphase-group, and (usually) divides in advance of the other chromosomes. These facts fully establish

¹³ I may point out that it is inadmissible to designate as '*m*-chromosomes' any pair of especially small chromosomes without respect to their other characteristics as has been done by several writers. The *m*-chromosomes of the Coreidae are not always distinctly smaller than the other chromosomes, and they are characterized by certain very definite peculiarities of behavior. Cf. Wilson, '05 c, '11 a.

the identity of the sex-chromosomes in *Oncopeltus*. It may therefore be taken as an established fact that a pair of sex-chromosomes may be recognizable as such even in cases where they show no perceptible difference of size, and where no constant differences between the diploid chromosome-groups of the two sexes can be seen.

Such cases are of course fatal to the view that the nuclear differences between the sexes are reducible to one of general chromatin-mass; and, as I have elsewhere urged, these chromosomes can be regarded as factors in sex-production only by assuming some kind of difference between the substance of *X* and *Y*. I will not here enter upon the discussion of a point that has been fully considered in several earlier papers (see especially Wilson, '11 a, '11 b). I will only again express the view that the differential factor between *X* and *Y* may plausibly be regarded a specific chemical substance (the '*X*-chromatin') that is either confined to the *X*-chromosome or is there present in relative excess, and in respect to which the two sexes differ correspondingly. If this is correct, the sexual differences may be at bottom dependent upon a fundamental quantitative difference of metabolism, as stated in my first paper on this subject ('05 a). Such nuclear differences between the sexes may of course exist not only in forms where no difference of total chromatin mass is visible, but even where no special 'sex-chromosomes' are differentiated. The surprising thing, indeed, is that they should in some instances be expressed in, or accompanied by, visible sexual differences of the chromosome-groups.

III. CRITICAL CONSIDERATIONS ON THE MATURATION-PHENOMENA BASED ON A COMPARISON OF THE HEMIPTERA, TOMOPTERIS, BATRACOSEPS AND SOME OTHER FORMS

As has been indicated, my conclusions concerning synapsis and reduction in Hemiptera are largely tentative in character. If I nevertheless venture to make some critical comment on the general problem it is mainly because of the opportunity I have had to reexamine these phenomena in *Tomopteris* and *Batrachoseps*.

1. The question of synapsis

The cytological problem of synapsis and reduction involves four principal questions, as follows: (1) Is synapsis a fact? Do the chromatin-elements actually conjugate or otherwise become associated two by two? (2) Admitting the fact of synapsis, are the conjugating elements chromosomes, and are they individually identical with those of the last diploid or pre-meiotic division? (3) Do they conjugate side by side (parasynapsis, parasynsidesis), end to end (telosynapsis, metasynsidesis) or in both ways? (4) Does synapsis lead to partial or complete fusion of the conjugating elements to form 'zygosomes' or 'mixochromosomes,' or are they subsequently disjoined by a 'reduction-division?' Upon these questions depends our answer to a fifth and still more important question, namely, (5) Can the Mendelian segregation of unit-factors be explained by the phenomena of synapsis and reduction?

Despite the prodigious accumulation of data regarding these questions the unprejudiced student of the literature finds himself compelled to admit that not one of them has yet received a really demonstrative answer—at least not one that has brought conviction to the minds of all competent cytologists. I do not propose to consider them exhaustively, or to give any approach to a complete review of the literature. This has been done by other writers, notably by Grégoire ('05, '10) in two extended and masterly memoirs, by Strasburger in a most valuable series of critical essays ('07, '08, '09, '10), and by Haecker ('07, '10). (See also Davis, '08, Gérard, '09, Gates, '11, Montgomery, '11, and the series of papers by the Schreiners and by Janssens.) I will however indicate some of the conclusions to which I have been led in an effort to form an independent judgment concerning the facts, especially in *Tomopteris* and *Batrachoseps*, which are probably unsurpassed as objects of observation, have become classical through the well known studies of the Schreiners ('06, '08) and of Janssens ('03, '05), and have formed a main center of controversy in recent years.

The conclusions of these observers (more especially those of the Schreiners) have been the object of repeated criticism on the part of Goldschmidt ('06, '08), Fick ('07, '08), Meves ('07, '08, '11), Haecker ('07, '10) and many others. These criticisms, too well

known to call for extended review, were substantially at one in the contention that what had been described as a parallel or sidewise conjugation of spireme-threads during the 'bouquet,' 'synaptene' or 'amphitene' (synaptic) stage is nothing other than a modified form of longitudinal splitting, in which double threads, longitudinally divided from the beginning, are progressively differentiated out of the nuclear substance from one pole of the nucleus towards the opposite pole. In the course of his able critique Meves ('07) endeavors to break down the distinction between such a process and that which is seen in the prophases of somatic cells, contending that in both cases the longitudinal duality is brought about by a biserial grouping of the chromatin-granules of the resting nucleus, and urging that the process seen in the amphitene-nuclei is of essentially the same nature as the early division of spireme-threads in the diploid nuclei long ago described by Flemming. Unquestionably, this objection is worthy of the most attentive consideration, especially in view of the conclusion of several recent observers (considered more in detail beyond) that the longitudinal division of the spireme-threads is in some cases already in evidence in the chromosomes of the preceding anaphases or telophases, and that the two halves thus arising may separate more or less widely before the nuclei have entered the 'resting' state. For Meves there is no problem of synapsis. The Gordian knot is cut with the statement, "Die Geschlechtszellen bezw. ihre Kerne haben nach meiner Vorstellung (1907) die besondere Eigenschaft ererbt, beim Eintritt in die Wachstumsperiode nur die halbe Zahl von Chromosomen auszubilden" ('11, p. 296). Certainly the adoption of this simple solution would save a great deal of trouble; but I fear that the facts compel us to take a more roundabout way out of our difficulties. Goldschmidt and Haecker, on the other hand, do not doubt the fact of synapsis, and take issue only with the parasynaptic mode of conjugation. Concerning the latter Haecker's latest expression of opinion is as follows:

Vielmehr hat sich in mir . . . die Ueberzeugung befestigt, dass der Eindruck einer Parallelkonjugation im wesentlichen durch die teilweise Koïnzidenz zweier voneinander unabhängiger Erscheinungen

hervorgerufen kann, nämlich erstens eines mehr zufälligen oder, besser gesagt, *selbsterständlichen teilweisen Parallelismus der Fäden*, wie er durch die in der Synapsisphase bestehende *polare Anordnung* der Kernsubstanzen bedingt wird, und zweitens einer *verfrühten*, bei den einzelnen Objekten und Individuen je nach dem physiologischen und Konservierungszustand bald früher, bald später, bald regelmässiger auftretenden primären *Längsspaltung* ('10, p. 185).

Without citing other zoölogical critics at this point, attention may be called to the increasing tendency now apparent among botanical cytologists to reject, or at least to restrict, the theory of parasynapsis held by Strasburger, Allen, Berghs, Grégoire and a large number of other botanical "zygotenists," in favor of a telosynaptic conception like that of Farmer and Moore ('05), itself essentially like that many years earlier maintained by Haecker and Rückert among zoölogists. Among these may be mentioned Mottier ('07, '08), Gates ('08, '11), Davis ('09, '11) and Digby ('10). These observers and others, though differing more or less as to the details, are in agreement on the essential point that in some species at least the synaptic connection of the chromosomes is end to end, not side by side; and that a longitudinal duality of the spireme-threads at the synaptic period (synizesis, or earlier) is either absent, or if present is due either to an accidental parallelism or to a longitudinal splitting comparable to that seen in the diploid prophase. These observers are in substantial agreement that the chromosomes (if persistent entities) are originally arranged in linear series, and united end to end, in a spireme-thread which ultimately breaks apart into bivalent segments, each consisting of two chromosomes in parasynaptic union. The sidewise pairing, which undoubtedly occurs in some plants, is believed by Farmer and Moore, Mottier, and others to result from a secondary looping of these segments, which takes place long after the synizesis stage. Gates, however, expressly adopts the view that synapsis may take place by either method in different species, possibly even in the same species.

I must admit that my own faith in parasynapsis (such as it was) and even in synapsis itself, was materially shaken by some of the criticisms and observations that have just been indicated, and that I took up the study of the question in a distinctly scepti-

cal spirit. It was only after prolonged and repeated study of the same objects, in part of the same preparations, as those of the Schreiners and of Janssens, that this scepticism gave way to the belief that the conclusions of these observers (not to mention others) are probably well founded. I will not at this time publish new figures or photographs of these forms (of which I have a large number, particularly of *Batrachoseps*) but will here confine myself to a brief statement of the main reasons why I do not find it possible to accept the adverse criticisms that have been indicated.

There are two points that demand especial emphasis. One is the complete demonstration of the seriation of the stages that is afforded in the testis of *Batrachoseps*. The regular and panoramic progression of stages from the spermatogonial end of the testis to that of the spermatids renders error on this point out of the question; and in particular, there is no possibility of confusing the post-synaptic with the pre-synaptic stages, or the synaptic nuclei with those of the early prophase (pro-strepsinema) of the spermatocyte-divisions. The second point of importance is the essential accuracy of the figures of the Schreiners and of Janssens. Indeed, my only criticism of those of Janssens might be that the relations are often shown even more clearly in the preparations than in his figures, perhaps because the latter were in some cases made from material not quite as perfectly fixed as the best that has come under my observation. In the case of *Tomopteris* I have been able in a considerable number of cases to compare the figures of the Schreiners with the identical nuclei from which they were drawn. Here and there, perhaps, certain details might be somewhat differently represented by different observers; but a study of very numerous cells at every stage of the spermatogenesis has thoroughly convinced me that as a whole the figures of these authors present a faithful picture of what any observer may see in the preparations. The only question that can be raised seems to me therefore to be a matter of interpretation.

I think that any observer, whatever be his individual prepossession, who will take the trouble to study these preparations thoroughly, will find himself compelled to admit the following facts:

1. That the 'amphitene' stage (to employ Janssens's appropriate term for the synaptic nuclei) is preceded by one in which the nucleus is traversed by fine, undivided, leptotene threads the free ends of which are from an early period polarized towards the pole of the nucleus near which lie the centrioles.

2. That from this pole, during the amphitene stage, thick and often plainly double threads are formed progressively towards the opposite pole near which (in *Batrachoseps*) lies the chromoplast.

3. That *pari passu* with the growth of the thick threads the thin threads disappear until all have vanished.

The conclusion is irresistible, and will hardly be disputed, that the thick (pachytene) threads grow at the expense of material supplied by the thin ones (leptotene).

It is further indisputable that in many cases the thick (and often double) threads terminate anti-polewards in *two* undivided diverging thin threads like the branches of a *Y*, which often separate at a wide angle and may be traced for a long distance, sometimes to opposite sides of the nucleus, as continuous threads. This fact may be seen in both *Tomopteris* and *Batrachoseps* with a clearness that admits of no doubt. These *Y*-figures are so numerous, so clear, and in their more striking forms so different from anything seen at other stages as to constitute a highly characteristic feature of the nuclei at this particular stage.

Janssens, the Schreiners, Grégoire and others have with good reason insisted on the fact, seen with especial clearness in *Batrachoseps*, that *not more than two thin threads are thus seen diverging from the anti-poleward ends of the thick threads*. Fick ('07) after examination of the Schreiners' preparations of *Tomopteris*, stated that he could sometimes observe more than two such diverging threads. Even Janssens in his earlier work (with Dumez) on *Plethodon*, believed that he had seen a similar appearance. "Un chromosome naissant est parfois en relation avec plusieurs filaments, de tel manière qu'il devient très difficile à l'observateur de faire un choix" ('03, p. 423); but in his later work on *Batrachoseps* he insists that such is not the case.

I have studied this point with the greatest care of which I am capable in both *Tomopteris* and *Batrachoseps*. In the former

case one may indeed often be in doubt, particularly in the earlier stages, though many perfectly clear *Y*-figures are evident. Such doubtful cases may however very well be due either to a confusion produced by threads of linin, to defects of fixation, or to coagulation-products of the nuclear enchylema. *Batrachoseps* seems to me, however, decidedly more favorable for study of this point than *Tomopteris*, partly because of the much greater size of the nuclei, partly because of the greater brilliancy of the pictures in detail, especially evident in material fixed with Carnoy's fluid. At its best this method, in my experience, is much superior to Flemming's or Hermann's fluids for study of this point. Prolonged search among the huge amphitene-nuclei of *Batrachoseps* has failed to show even a single clear case in which more than two leptotene-threads can be traced into connection with a single pachytene. When the latter terminate anti-polewards in more than one leptotene-thread two are always seen, very often diverging like the branches of a *Y*; and these bifid figures appear with the utmost clearness in every view—sidewise, endwise and obliquely. It is of course true that such bifid figures are often not in evidence. Not infrequently pachytene-threads seem to end abruptly without connection with the leptotene; sometimes they seem to end in single leptotene-threads. But in the nature of the case the true relation of the latter to the former must often fail to appear in the sections. This may result from many causes—accidents of sectioning, entanglement of the threads, unfavorable position, and the like—and it is very probable that in the coagulation-process of fixation the delicate thin threads may often break away from their normal connections. When allowance is made for these sources of error it is in fact surprising that so many demonstrative *Y*-figures are seen; and it is a significant fact that these figures, though often bent or distorted, always show the same orientation in the nucleus with respect to the centrosome pole.

That the *Y*-figures represent the normal and typical relation of the pachytene-threads to the leptotene seems to me indisputable; and I consider it utterly impossible to interpret these figures as an expression of a progressive longitudinal splitting of previously

undivided threads. The Y-figures are not opening apart, they are closing up, as is placed beyond doubt by the magnificent demonstration of the seriation given in the testis of *Batrachoseps*. Y-figures with a short stem and long arms precede; they do not follow, those with long stem and short arms. What is taking place is evidently a coming together of the thin threads side by side in pairs to form the thick ones—a process exactly opposite to longitudinal division. I do not hesitate, therefore, to confirm positively the description of the *facts* given by Janssens and the Schreiners.

I desire to emphasize the striking contrast that exists between the amphitene-nuclei and the spermatogonial or other diploid prophase-nuclei. It seems to me that Meves goes much too far when he directly compares the process of 'parallel conjugation' to the early fission of spireme-threads in the diploid nuclei as described by Flemming and his successors; for one is led from this to suppose that figures may be seen in the two cases that are essentially similar. But no one can study the early spermatogonial prophases in *Tomopteris* or *Batrachoseps* without being struck by the very great contrast which they present to the amphitene-nuclei.¹⁴ Never in the former case, as far as I have been able to find, are the two halves of the double spireme-threads seen diverging like the branches of a Y; nor have I been able to discover such pictures in the early prophases of other diploid nuclei, such as the epithelial and connective tissue cells of larval salamanders. Even though such pictures could be found, the amphitene nuclei undeniably offer peculiarities that differentiate them in the most

¹⁴ In considering this question it is necessary to point out that the single figure of the amphitene stage that Meves offers in favor of his interpretation ('07, text-figure, p. 460) conveys no real idea of the characteristic relation of the leptotene-threads to the pachytene. Many pictures similar to this are seen in my own sections of *Batrachoseps* and *Plethodon*, especially after fixation by Flemming's fluid or Hermann's, often also in inferior preparations from Carnoy's fluid; but as a rule it is only in the best Carnoy preparations that the exact relations can be clearly and generally seen. It is evident that the least defect due to fixation or to the shrinkage of embedding process tends to obscure the leptotene-threads and cause them to assume a more netlike appearance.

conspicuous way from the earlier generations of cells in the testis; and these are not to be ignored in the study of this problem.

Another very striking fact in the case of *Batrachoseps* is that the two branches of the *Y* often give exactly the appearance of twisting together to form the stem—a condition very clearly shown in many of Janssens's figures, though I do not find it mentioned in the text. The pictures seen in *Tomopteris* also sometimes suggest a similar condition, though less clearly; but in neither case am I entirely sure of the case, since the torsion often can not be seen. A twisting together of the longitudinal halves of the diplotene-threads at a later stage ('strepsinema') is of course a very familiar fact; but I can find only a few indications here and there in the literature of such a twisting at the synaptic stage. Two very definite accounts of such a process have recently been given by Agar ('11) in the case of *Lepidosiren*, and by Bolles Lee ('11) in *Helix*. The latter author believes the double spiral to persist as such in the succeeding pachytene stage. "Jamais, à aucune moment, même dans les enroulements les plus étroits du bouquet tassé, on ne voit rien qui puisse faire conclure à une fusion des deux éléments" (p. 70). It is quite possible that a close torsion of the threads may explain the fact that it is in many cases difficult or impossible to distinguish a longitudinal duality for a certain time after the synaptic process is completed.

Concerning synapsis in the Orthoptera I can only speak with considerable reserve. Most observers of this group have concluded that the longitudinal duality of the diplotene-threads is due to a process of longitudinal splitting (McClung and all of his pupils, Sinéty, Montgomery, Davis, Buchner, Jordan, Granata, Brunelli) and only a few have attributed it to parasynapsis (Otte, Gérard, Morse). The few observations I have been able to make on McClung's preparations of *Achurum*, *Phrynotettix* and *Mermiria* nevertheless lead me to the impression that a side by side union of leptotene-threads takes place here also. The case is however much less clear than in the other forms since the polarization is less marked, and the amphitene stage, though clearly apparent in some cases, is correspondingly less conspicuous.

Nevertheless, in these nuclei also the leptotene-threads may often be seen to lie parallel and in pairs on one side of the nucleus, while on the opposite side they are quite irregular.¹⁵ Here too may be seen Y-shaped figures, in some cases almost exactly like those of *Batrachoseps*, except that the stem is more clearly double and shows no indication of torsion. It may again be pointed out that Sutton's observations on *Brachystola* are entirely consistent with a parasynaptic mode of conjugation. I think therefore that the case for telosynapsis in these animals is not yet established, and that in spite of the careful work of Davis, Brunelli and others, the question must still be considered open.

As to the Hemiptera, sufficient emphasis has already been laid upon the practical difficulties which they present. In *Euschistus* however, as described in Montgomery's recent valuable paper ('11) the difficulties are less baffling than in many other forms; and he has had the advantage of working with a species in which the total number of threads can be determined in at least some of the nuclei at every stage. In this work the author, reversing his earlier conclusions concerning these insects, definitely accepts the theory of parasynapsis. As I have pointed out, the prophase-figures in *Oncopeltus* and *Protenor* are certainly not out of harmony with this conclusion. I therefore accept the probability of a side by side union in these animals, though I think the possibility of an end to end conjugation is not yet excluded.

But if, now, the fact of a side by side union of parallel leptotene-threads be granted, we have still not arrived at a demonstration of parasynapsis; for there are some very important possibilities yet to be reckoned with. Before such a demonstration can be admitted, we must first make sure of the number of separate pre-synaptic chromosomes (cf. Fick, Meves) and secondly must exclude the possibility, which has been suggested by several writers, that the parallel union is no more than a reunion of sister-threads that have been derived by an earlier longitudinal fission of a single thread (or chromosome) and have subsequently

¹⁵ This was also noted by Davis but attributed by him to "an accidental arrangement, which is more common near the pole since in this region the threads are crowded more closely together" ('08, p. 127).

STUDIES ON CHROMOSOMES

undergone wide separation. Such, for instance, is the view of Brunelli ('11) in an interesting recent work on *Tryxalis*; and a similar view is suggested on the botanical side by the recent work of Digby ('10) on *Galtonia*, and of Fraser and Snell ('11) on *Vicia*. All these observers believe the longitudinal duality of the spireme threads at the synaptic period to be quite comparable to that of the diploid prophase, and to be traceable to a longitudinal split that is already present in the preceding telophase-chromosomes (cf. also the work of Dehorne and of Schneider, already cited), and these writers emphasize the fact that the products of this fission do in fact separate more or less widely as the nuclei enter the 'resting' period. As to the subsequent changes Brunelli concludes, "Successivamente, le due metà longitudinale degli individui cromosomici si parallelizzerebbero: donde gli aspetti intermedi che sono stati descritti come scissione di un filo unico, o come l'accollimento di due fili cromatici avendo il valore di due cromosomi (ipotesi della zigotenia)" ('11, p. 9). It is evident from this how essential it is to determine the number of pre-synaptic threads; for if they have such an origin as has just been indicated, their number should be tetraploid (double the diploid), whereas if they represent whole chromosomes the number should be diploid.

In the insects that I have studied the pre-synaptic stages are of especial interest as affording almost a demonstration that the pre-synaptic number of threads is the diploid number. I attach great weight to the history of the sex-chromosomes in these stages; for, owing to the fortunate circumstance that they are individually recognizable at this time, we can be perfectly sure that at least one pair of chromosomes of the diploid groups is here represented by two separate chromosomes that afterwards undergo synapsis. When we consider that these chromosomes are hardly distinguishable from the other chromatic masses of Stage *b* until after considerable extraction, that the latter are of the same or nearly the same number as that of the spermatogonial autosomes, and that they give rise to the separate leptotene-threads that enter synapsis, we must admit that strong ground is given for the conclusion that the latter are individually representatives of

the spermatogonial autosomes. In some at least of the objects I have examined these threads are single, not double; and I can find no evidence that they consist or have consisted of two interlacing spirals or closely associated halves. In this respect they seem to be quite like the spirals that uncoil from massive bodies in the spermatogonial prophases in *Phrynotettix*. In this case I can speak with complete assurance; for the evidence afforded by McClung's brilliant preparations of this form is absolutely demonstrative that the spirals are single, and that the longitudinal duality is produced by a subsequent longitudinal split of the spiral thread (which is essentially in agreement with Janssens's earlier conclusions in the case of *Triton*).

For the foregoing reasons I accept the probability that the parallel union of leptotene-threads does not form part of a peculiarly modified process of longitudinal division, but should be regarded as a true conjugation or parasynapsis of entire chromosomes. Apart from the convincing evidence afforded by the sex-chromosomes, my observations are essentially in agreement with those of the Schreiners in regard to the origin of the leptotene-threads. These observers describe the latter in *Tomopteris* as arising from much thicker, loose, polarized loops of the *diploid number* (18) which transform themselves into convoluted threads in a manner somewhat similar to that seen in the insects: "Nicht selten haben wir Bilder gesehen, die uns den Eindruck gegeben haben, dass das Chromatin der lockeren Schlingen sich zuerst zu einem unregelmässig aufgebauten, stark gewundenen und gefalteten Bande sammelt, aus dem wieder die deutlich begrenzten dünnen Fäden hervorgehen" ('06, p. 18). Later, "Die Chromatinfädchen, die auf Stadien wie Fig. 18 und 20 *a* hervortreten sind, wovon uns fortgesetzte Untersuchungen immer fester überzeugt haben, in den breiten aufgelockerten Chromatinbänder der vorgehenden Stadien *spiral aufgerollt oder zusammengefaltet sind*" ('08, p. 10, italics mine). My own study of the *Tomopteris* slides gives me the same impression; and I think it probable that the phenomenon here seen is of the same nature as that which so clearly appears in the insects, though the thick 'Chromatinbänder' are here much less sharply defined. Jans-

sens's somewhat similar account of the pre-synaptic stages in *Triton* have already been mentioned (p. 368). In *Batrachoseps*, on the other hand, there is as yet nothing to show that the leptotene-threads arise directly from the irregular and variable chromatin-masses that are seen in the earlier stages ('protobroch' and 'deutobroch' nuclei).

Opinion still differs so widely in respect to the pre-synaptic conditions in plants that its discussion can hardly be undertaken here. Overton ('05, '09) and those who have adopted the 'prochromosome-theory' find the leptotene stage preceded by one in which massive 'prochromosomes' are present, of the diploid number, and already showing an association in pairs which is a forerunner of actual synapsis; but other observers have found no support for this view in the objects they have examined (cf. Mottier, '07, '09). It seems possible that different species may differ in this respect, as is certainly the case in animals.

It is impossible to leave this discussion without mention of two additional series of facts which lend strong indirect support to the theory of synapsis. One is the remarkable discovery that in the diploid groups the chromosomes are often found to correspond two by two in respect to size, as was first pointed out by Montgomery ('01) and Sutton ('02); and that in some cases the chromosomes are actually arranged in pairs according to their size. The latter fact was also first described, I believe, by Montgomery ('04) in *Plethodon*, later in a number of the Hemiptera ('06); and in the latter work first appears the view that such an actual arrangement in pairs is characteristic of the diploid nuclei (p. 148). A similar arrangement was later described by Janssens and Willems ('08) in *Alytes*; and a most striking, unmistakable case of the same kind was found by Stevens ('08) in several of the Diptera. On the botanical side similar facts have been described by Strasburger ('05), Geerts ('07), Sykes ('09), Müller ('09), Gates ('09), Stomps ('11) and others. Overton, Rosenberg, Lundegard, and others likewise describe the "prochromosomes" as arranged in pairs in the diploid nuclei, as well as in the pre-synaptic stages of the auxocytes. So many of these cases have been described, some of them of quite demonstrative character

(Diptera), that no doubt can exist of the widespread *tendency* of the chromosomes to assume this arrangement already in the diploid nuclei. It appears to me however that those authors who consider the paired grouping to be a general characteristic of the diploid nuclei go much too far.¹⁶ Not only are numerous exceptions seen in the case of individual chromosome-pairs, but in many cases no trace of paired grouping appears. Such exceptions may readily be seen in the figures of Montgomery, Stevens, Morrill and myself of the Hemiptera, which are probably unsurpassed among animals for the clearness with which the size-relations are shown. In cases where certain pairs may be unmistakably recognized (as in *Protenor* and other *Coreidea*) the two members frequently show no constancy of relative position—compare for instance the accurate figures of the diploid groups of *Protenor*, *Anasa*, *Alydus* and *Nezara* in my third 'Study' ('06) or those of Morrill ('10) of *Archimerus*, *Chelinidea*, *Anasa* and *Protenor*.

But this does not in the least lessen the significance of the remarkable cases that have been established. The tendency towards such an association of the chromosomes in pairs is undoubtedly widespread; and the very fact that it does not follow a fixed order may be used as an argument in favor of a conjugation at the synaptic period. When the pairing is already evident in the diploid groups the way for synapsis has been prepared in advance. This process must take place at some time subsequent to the association of the germ-nuclei in fertilization; and that such a process undoubtedly occurs nullifies all the a priori objections that might be urged against the possibility of a corresponding process that is delayed until the maturation-period is reached.

¹⁶ Strasburger, for example, says, "Ich zweifle nicht im geringsten daran, dass es sich um eine allgemeine Erscheinung in diploiden Kernen dabei handelt, wenn sie auch nicht immer auffällig ist" ('09, p. 90). Gates is still more specific. "It is evident that the pairing of the chromosomes is not brought about at synapsis or at any other period of meiosis, but that the chromosomes are really paired throughout the life cycle of the sporophyte . . . Synapsis plays no special part in the pairing . . . Meiosis and reduction consists essentially in the segregation of the members of these pairs that have been in association since soon after fertilization" ('11, pp. 334, 335).

Lastly may be mentioned the interesting facts observed in the maturation of hybrids between parental forms having different numbers of chromosomes. Well known as Rosenberg's results on *Drosera* are ('04, '09), the main facts may be again outlined, especially as exactly analogous results have recently been reached by Geerts ('11) in hybrid *Oenotheras*. On crossing *Drosera longifolia* (having forty chromosomes) with *D. rotundifolia* (having twenty chromosomes) the hybrids have the intermediate number of chromosomes, thirty ($20 + 10$). In the first maturation-division appear ten double and ten single chromosomes, the former undergoing a regular division and distribution to the poles, while the latter fail to divide, undergo an irregular distribution, and often fail to enter the daughter-nuclei. Rosenberg's interpretation is that the ten *rotundifolia* chromosomes conjugate with ten of the *longifolia* ones to form the ten bivalent (double) chromosomes, leaving ten *longifolia* chromosomes as unpaired univalents which undergo irregular distribution. The results of Geerts are exactly analogous. *Oenothera gigas* (twenty-eight chromosomes) crossed with *Oe. lutea* (fourteen chromosomes) gives hybrids with twenty-one chromosomes ($14 + 7$). The first division shows seven double (bivalent) and seven single (univalent) chromosomes; and, as in *Drosera*, the bivalents divide equally and symmetrically, while the univalents wander irregularly along the spindle and often fail to enter the daughter-nuclei. His interpretation is the same as that of Rosenberg.¹⁷ If correct, these results, indirect though they be, constitute almost an experimental demonstration of both synapsis and reduction.

In summing up, it is my opinion that in spite of all the apparent contradictions and conflict of opinion concerning the *modus oper-*

¹⁷ Gates however ('09) in an earlier study of the same hybrid examined by Geerts, was led to quite different results, concluding that half the pollen cells receive 10 chromosomes and half 11. I can not, however, find evidence in his paper to sustain his conclusion that "there is not here a pairing and separation of homologous chromosomes of maternal and paternal origin" (op. cit., p. 195). Gates gives no account of the exact mode of distribution of the chromosomes in the heterotypic division, but only the end result. This result would however follow if exactly such a pairing and disjunction took place as is described by Geerts, provided the remaining chromosomes also underwent an approximately equal distribution. It remains therefore to be seen whether the apparent contradiction of results is real.

and of synapsis, the cumulative force of the evidence in favor of the fundamental fact is irresistible. This question is not to be judged alone by the study of any one of its single phases. The whole extensive series of facts must be reckoned with; and despite their variations in detail the data are too consistent in their fundamental aspects, to be explained away. On the other hand, it is obvious that the problem as to how the parental chromatin-homologues become definitely associated in pairs is still far from a definitive solution. We should certainly expect a phenomenon so fundamental to follow everywhere the same type; but I am in agreement with the opinion that has been expressed by some other writers (cf. Gates, '11) that the particular mode of union may be of subordinate significance. In accepting the main conclusions of the Schreiners and of Janssens in regard to *Tomopteris* and *Batrachoseps* I do not mean to imply that end to end conjugation, or telosynapsis, may not also take place in other forms. I hold no brief for parasynapsis; and I fully recognize the weight of the evidence in favor of telosynapsis recently brought forward especially by the botanical cytologists that have been cited. The studies of King ('07, '08) on *Bufo* should also be emphasized in this connection. I repeat that the phenomena seen in the insects by no means exclude the possibility of synapsis of this type, at least in some forms. Nearly all observers are agreed that the side by side union begins at or near the free ends of the leptotene-threads and advances step by step along their course. We can here see how readily the one mode might pass into the other; and the suggestion of Gates that the mode of synapsis may be correlated with the shape of the conjugating elements at the time of their union seems well worthy of consideration.

It is not to be denied that the acceptance of parasynapsis involves some very puzzling difficulties. It is, for instance, hard to comprehend how long loop-shaped chromosomes can become so disposed as to undergo progressive side by side union from their free ends towards the central points, as both the Schreiners and Janssens have concluded. Janssens appears to recognize this when he says: "*L'éloignement des filaments (i.e., the wide divergence of the branches of the Y-figures) avant leur soudure est un*

fait très étonnant, mais absolument certain;" but he very justly adds, "De ce que nous ne pouvons pas expliquer par quel mécanisme la soudure se réalise dans de tels cas, il ne s'en suit rien contre son existence indubitable" ('08, p. 167). However difficult such a mode of union may seem a priori, the preparations of the Schreiners actually demonstrate double loops that are united at both ends while widely separated along their middle portions—shown for example in figs. 16, 17 and 18 of their paper of 1908, which accurately represent the facts, as I am able to confirm from examination of these identical nuclei in the original preparations. We must seek to discover by observation how the conjugating loops disentangle themselves from the apparent chaos of the leptotene-spireme. The chaos may however be apparent rather than real. The interesting facts worked out by Janssens in regard to the persistent orientation of the loops in the pre-synaptic stages of *Batrachoseps* indicate that their polarity is not lost at any time between the final spermatogonial anaphases and the amphitene stage, and that their free ends always converge towards the centrosome. It seems quite possible that the way for synapsis may be prepared already in a very early pre-synaptic stage, by a definite regrouping of the chromosomes that may take place before the leptotene loops are formed as such. It is evident that the central portions of the loops are constantly shortening as the peripheral portions come together (possibly as a result of the progressive torsion of the latter). It seems therefore by no means a hopeless task to undertake a definite solution of the puzzle by observation.

2. *The question of the reduction-division*

The history of the sex-chromosomes in *Oncopeltus* affords, I believe, complete demonstration of the occurrence both of synapsis and of a true reduction-division in the original sense i.e., of the disjunction of two entire chromosomes that have previously conjugated in synapsis. But, although this creates a certain presumption in favor of the occurrence of a similar process in case of the other chromosome-pairs, this argument must not be pushed too far—indeed, there is reason to believe that in case of the ordi-

nary chromosomes ('autosomes'), the process may be different in some important respects from that seen in the sex-chromosomes; and we must not lose sight of the wide difference of behavior in other respects that differentiates the latter from the former. It is possible that the sharply marked process of conjugation and disjunction characteristic of the sex-chromosomes and *m*-chromosomes may be correlated with their specific functional relations. The case for the autosomes must therefore rest upon their direct study, and a reduction-division can only be fully established by tracing the bivalents as double bodies or 'gemini' through every stage from the time of their conjugation to that of their disjunction.

Whatever view of synapsis be adopted, this is a difficult task. If we take the view that the chromosomes are arranged in linear series in a spireme-thread which breaks into bivalent segments each consisting of two chromosomes in telosynaptic union there is no guarantee, as far as I can see, that the latter ultimately separate at the synaptic point. If we accept parasynaptic conjugation the difficulty is of a different kind, namely, the extremely close union of the conjugants side by side, which as nearly all observers are agreed, follows upon synapsis. The most that has been asserted by these observers has been that evidence of longitudinal duality can always be seen in some of the bivalents at every stage. Without reviewing all these cases, I will only recall that in the case of *Batrachoseps*, for example, Janssens says, "Pendant le long stade du bouquet, les anses sont simples et par aucune méthode cytologique nous ne parvenons à y reconnaître la moindre trace de dualité" ('05, p. 401). In case of *Tomopteris* the Schreiners admit that at a period shortly following synapsis no longitudinal division can be seen in the pachytene-threads; and these authors are compelled to fall back upon indirect evidence in support of their conclusion that the duality is not really lost. Again, in Montgomery's recent work on *Euschistus* ('11) he expresses the conviction that there is no valid evidence of any actual fusion between the conjugants; yet in point of fact states that after the completion of synapsis "The autosomal loops (bivalents), are in one-half the normal number and, for the most

part, each of them appears solid and undivided." The most that can be said appears to be that "in the greater number of cases there is to be seen in each geminus at least traces of a clear space which marks the original point of meeting of two univalents" ('11, p. 738). It seems to me that this is hardly a sufficient basis for so important a conclusion. Many similar statements might be cited from other authors. On the other hand some very competent observers not only find no evidence of duality in the early post-synaptic bivalents but definitely conclude that the conjugants completely fuse to form 'zygosomes' or 'mixochromosomes' (e.g., Vejdovsky, '07, for the Enehytridae, Bonnevie, '08, '11, for *Allium* and other forms, Winiwarter and Saintmont, '09, for the cat).

In the case of *Batrachoseps* I can fully confirm Janssens's statement that no evidence of longitudinal duality can be seen in the pachytene-loops throughout the greater part of the growth-period, even in the most perfect preparations, and after various modes of fixation, staining and extraction. It is only in the earlier period that the duality appears, and then often only here and there and in a small portion of the thread. It is the same in *Tomopteris*. The longitudinal cleft often so clearly seen at the time of synapsis seems soon to disappear, so that for a time nothing can be seen in the pachytene-threads to indicate their bivalent nature. Theoretically it is of course quite possible that this appearance is deceptive, and that the two elements are in reality always distinct; but if we resort to theory an equally strong case can be made out, I think, in favor of partial or complete fusion. It seems at any rate certain that in some of the most favorable material thus far found among animals, synapsis is followed by a union so intimate that no adequate evidence of duality is afterwards seen until the diplotene stage is reached in the prophase of the first division. It is very possible that this may be due in some cases to a close twisting together of the threads (cf. p. 399) but it would hardly be safe to accept such an explanation at present.

I am myself inclined to accept the evidence at its face value, and to conclude that parasynapsis is followed by at least a partial

fusion of the two conjugants; and that the synaptic process involves not merely an association of the chromosomes to form 'gemini' but a process of reconstruction which may profoundly change their composition (cf. Boveri, '04). I am, however, by no means in agreement with those writers who for a similar reason would reject in toto the conception of the reduction-division in the case of these chromosomes. Very important evidence upon this point is afforded by the contrast in structure and behavior between bivalents and univalents in the maturation-divisions; and this has not yet received sufficient attention on the part of writers on this general subject. In the first place, it is a rule, without exception so far as I am aware, that univalent chromosomes divide but once (of course equationally) in the course of the two maturation-divisions, while bivalents divide twice. The additional division in case of the bivalents must, therefore, be in some manner a consequence of synapsis. In the second place, the difference between univalents and bivalents is often clearly displayed in a characteristic difference of structure in the prophase. In the insects that I have studied the former are always bipartite bodies, the latter often quadripartite—obviously in preparation for a single division in the former case, for two divisions in the latter. The best examples of these facts are offered by the sex-chromosomes; but they are also exhibited by the *m*-chromosomes of Hemiptera, and by certain anomalies sometimes seen in the autosomes.

Perhaps the most striking of these cases is that of the X-chromosome because of the different conditions seen in different species. In some forms this chromosome is accompanied by a synaptic mate (the Y-chromosome) with which it unites to form a bivalent before the spermatocyte-divisions (Coleoptera, Diptera); in other species X and Y divide as separate univalents in the first division and afterwards conjugate (many Hemiptera); while in still others Y is missing and X is always univalent. In the first case the XY-bivalent 'divides' in both spermatocyte-divisions—reductionally in the first, equationally in the second, as may be clearly seen because of the inequality of X and Y (Stevens). In the second case (e.g., *Oncopeltus Lygaeus*) this order is reversed, the first division being of course equational, the second reductional. In

the third case *X* divides in but one spermatocyte-division (either the first or the second according to the species) and in the other passes undivided to one pole. It is here perfectly clear, as has been urged by McClung ('01, '02) and myself ('05 c), that the failure to divide in one division is due to the absence of a synaptic mate; and it is thus rendered doubly certain that in case of the *XY*-pair but one true division (an equational) takes place, the other 'division' (reductional) being merely the separation of the synaptic mates. This is, I think, a conclusive demonstration in the case of these chromosomes of the reality (1) of the conception of bivalence, (2) of the reduction-division in its original and unmodified sense. That these conclusions are not limited to the sex-chromosomes is shown by the *m*-chromosomes of the Hemiptera, which have no relation to sex as far as known. In this case conjugation (synapsis) is usually delayed until the last possible moment before the first division. Their separation, which immediately ensues, is again a true reduction-division of the *m*-bivalent; but what we here call a 'division' is obviously not properly such but only the disjunction of two distinct bodies that have but just come into momentary contact. In this case, as in that of the *XY*-pair, the term reduction 'division' is a misnomer. But one actual division takes place, the equation-division. This is fully borne out by the interesting anomaly that I described in a single individual of *Metapodius* in my sixth 'Study,' consisting in the presence of three *m*-chromosomes instead of two. Here all three uniformly couple to form a triad element in the first division, which immediately breaks up into its components, of which one passes to one pole and two to the other. In the second division all three divide equationally, so that half the spermatids receive but one *m*-chromosome half two. This is, of course, exactly in accordance with expectation; and it is a remarkable fact that the two *m*-chromosomes that are present in half the secondary spermatocytes do not disjoin but divide equationally, as they should.

The case of the autosomes is different, owing to the intimate union and possible fusion that follows synapsis; and it seems probable that the reduction-division must here be regarded in a differ-

ent light. The history of these chromosomes as contrasted with that of those just considered, nevertheless affords some important evidence bearing on this question. As has been stated, the prophase-bivalent is of quadripartite composition (though this may fail to become visible until the later prophases) while the prophase-univalent is bipartite. This has already been emphasized in the case of *Oncopeltus*, but may be studied to still greater advantage in *Protenor*, because of the greater size of the chromosomes and of their very marked individual size-differences. In the male of this form, as heretofore described by Montgomery, Morrill and myself, the spermatogonial groups contain thirteen chromosomes, and the unpaired *X*-chromosome is very nearly twice the size of the largest pair of autosomes (photos. 35, 36). In the female two such *X*-chromosomes are present (photos. 37, 38). In the male the *X*-chromosome of course remains univalent throughout the entire maturation-process, while the large pair of autosomes produces a bivalent that is of nearly the same size as the univalent *X*. The latter is at every period distinguishable. In the earlier stages of the growth-period, when the autosomes are in a diffuse and lightly-staining condition, it remains compact, in the form of a somewhat elongate vermiform body, that is closely coiled about or within a plasmasome to form a rounded chromosome-nucleolus the true nature of which only appears after considerable extraction (cf. Montgomery, '01, fig. 127). In smears (as is the rule among the Hemiptera) the plasmasome usually collapses, setting free the *X*-chromosome, when its rod-like form becomes clearly apparent (photo. 39). In later stages it shortens, thickens and splits lengthwise, so as to appear in the prophases as a rather short, thick rod, very plainly split (figs. 120-131, photos. 40 to 51). The large bivalent, on the other hand, first becomes recognizable in the prophases, as the process of condensation occurs, when it may be studied to the best advantage in smear-preparations, in which all the chromosomes are spread out in one plane.

In such preparations, of which I have a large number, the large bivalent is invariably distinguishable by its large size—nearly twice that of any of the others; and we thus have opportunity to compare it accurately, *side by side in the same nucleus*, with a

univalent chromosome (X) of the same size. It is most interesting to observe in *Protenor* the gradual emergence of the bivalents from the confused nuclear threadwork of Stage *g*. Early stages of the process are seen in figs. 115 to 117, which clearly demonstrate (1) that the threads do not constitute a continuous spireme, but are separate, (2) that they do not lie side by side in pairs to form a diplotene, but are single and undivided. Figs. 118 and 119 show two nuclei, only a little later than the preceding, in which all the bivalents are clearly seen and the large one is perfectly evident. It is of course only now and then that a nucleus in this stage can be found in which all the chromosomes are thus clearly distinguishable; the bivalents are still so extended and irregular as to present a hopelessly confused picture in sections, and very frequently also in smears. It is however my firm belief that the bivalent-figures, intricately entangled though they are, are already quite distinct at least as early as fig. 115, and I do not hesitate to accept this as probable for the still earlier and more confused nuclei that precede.

From the latter part of Stage *h* (i.e., figs. 118, 119) every step may readily be followed as the chromosomes continue to condense, contract and increase in staining capacity. Of the innumerable nuclei showing these stages in my smear-preparations a few are shown in figs. 120 to 131, and in photos. 40 to 51. As these figures show, the typical number of separate chromatin-bodies is eight, which may however be reduced to seven by the coupling of the two smallest (*m*-chromosomes, figs. 124, 129, 130, photos. 45, 46, 48), or in certain rare abnormalities may be increased to more than eight (fig. 128, photo. 43). Of these eight, three are univalent, namely, the two smallest (*m*-chromosomes) and the large X -chromosomes, the latter always distinguishable by its more compact consistency, greater staining capacity, rod-like form, and simple longitudinal split.

The remaining five are the bivalent autosomes, which from the beginning have the same forms as in *Oncopeltus*—i.e., double crosses, double *V*'s, or longitudinally split rods which sooner or later develop a transverse suture at their middle points and thus are plainly seen to be of quadripartite nature.

A particular interest attaches to the striking and constant contrast between the *X*-chromosome and the large bivalent; I do not here refer to their conspicuous difference in texture and staining capacity in the earlier stages but to a characteristic difference in morphological composition that persists up to the very metaphase of the first division. The *X*-chromosome is always bipartite—a simple rod, longitudinally split. Sometimes it is curved, sometimes slightly constricted at the middle point, sometimes irregular in form; but many of these variations are evidently quite accidental. It never shows the least approach to the double cross form nor does a transverse suture at the middle point make its appearance. In the final prophase it enters the spindle at right angles to the latter, and undergoes a longitudinal division (figs. 132 to 134, cf. Montgomery, '01, '06, Wilson, '11 d). The large bivalent, on the other hand, is always, sooner or later, a quadripartite body. In most cases it forms a fine double cross, of the same type as that already described in case of *Oncopeltus*, and showing the same variations. All gradations are found, in different nuclei, on the same slide, between forms in which the arms of the cross are equal (figs. 120, 122, 123, photos. 40, 41, 49) and those in which one pair (the 'lateral' arms) are but just perceptible (figs. 121, 124, 125). In some cases, comparatively rare, the lateral arms are quite wanting, and the bivalent appears as a longitudinally split rod (figs. 126, 129, 130) but in these forms a distinct transverse cleft or suture is often seen at the middle point; and in a few cases the rod is sharply bent at this point to form a *V*-shaped figure (fig. 127). Sometimes the transverse cleft is not seen in the earlier stages: but always in the later pro-phases it becomes evident (figs. 129, 130, 131, photos. 48, 51). In these stages, as in *Oncopeltus*, the lateral arms of the double crosses sooner or later disappear, being apparently progressively drawn in to the axial arms, and in their place appears first a transverse suture and later a constriction across which the first division takes place. In the early metaphases the large bivalent shows two extreme types, connected by all intermediate transitions. At one extreme are ring-like tetrads (fig. 133) which are evidently derived from crosses by shortening of the arms and per-

sistence of the central opening. At the other extreme are short tetrad-rods (fig. 132) which clearly show two division-planes at right angles to each other. These forms may be derived either from the more elongate rod-like forms of earlier stages or from crosses by disappearance of the lateral arms. In both types the quadripartite structure is unmistakable. The large bivalent ultimately assumes a dumb-bell form, with its longer axis parallel to that of the spindle-axis, and undergoes a 'transverse' division; while, as already stated, the X-chromosome always enters the spindle with its long axis transverse to that of the spindle, and undergoes longitudinal division (figs. 132, 134; see also Wilson, '11, fig. 9, Montgomery, '01, figs. 136, 138).

No observer who studies these nuclei attentively can fail to be struck by the remarkable difference between the large bivalent and the large X-univalent. Its explanation is obvious; the former is preparing to divide twice, the latter once, in the course of the two maturation-divisions. But this does not yet touch the root of the matter. We have still to ask why two chromosomes of equal size in the same nucleus differ so widely in respect to their mode of division. The reply is again obvious. It is because one of them has double the chromosomal value (or valence) of the other, the bivalent representing two chromosomes of the original diploid groups, while the univalent represents but one. This conclusion, which is hardly more than a statement of fact, is confirmed by a very interesting anomaly shown in fig. 128, and in photo. 43. This nucleus contains nine chromosomes, and the large bivalent is absent as such, while in its place appear two separate and equal chromosomes of half its size (*B, B* in the figure), while the four smaller bivalents are plainly recognizable (*b-b*). The explanation obviously is that through an abnormality of synapsis the two members of this particular pair have failed to unite and have therefore remained univalent. *Both of these chromosomes have the form of simple, longitudinally divided rods, without trace of a quadripartite structure*, while the four smaller bivalents all show the cross-form (though the lateral arms are but slightly developed in one of them). It may be surmised, I think, that if the later history of these separate univalents could be followed out, they would be

found to divide but once (equationally) in the course of the two spermatocyte-divisions, as in case of the *X*-chromosomes, or the *m*-chromosomes.

When these facts are taken together the conclusion seems to me unavoidable that one of the divisions of the bivalent chromosome (and hence, one of the division-planes seen in the bivalent prophase-figures) *is a consequence of its bivalence*—i.e., of its origin from two chromosomes instead of one, of the original diploid groups. An almost conclusive demonstration of this is given by the fact that when the *X*- and *Y*-chromosomes are united to form a bivalent in the prophases, this body, like the others, often shows a tetrad structure (as in *Brochymena*, Wilson, '05 b, or *Nezara*, Wilson, '11 a); and in the case of *Ascaris felis*, recently described by Edwards ('11) this bivalent has a double cross-form, closely similar to that of the other bivalents save for the inequality of two of the components (op. cit., fig. 2). I do not mean to imply that either division-plane of the tetrad represents the actual plane of separation of the same two chromosomes that have united in synapsis; on the contrary, I think it probable, as already indicated, that the original chromosomes may have undergone reconstruction. What may be said is that one division is independent of bivalence, the other a consequence of it; and it is further clear that the former effects no reduction of valence, while the latter does. Whether we regard the autosome-bivalent as to its origin or its fate, it has, irrespective of its relative size, double the chromosomal value of a univalent in the maturation-process; and in this respect it is exactly comparable to an *XY*-bivalent or an *m*-bivalent, in which one of the divisions is demonstrably a reduction-division in the original sense. This value, or 'valence' is reduced to one-half in one of the maturation-divisions. May we not here find a definition of the reduction-division that may be accepted even by those who deny the individuality of the chromosomes, or who believe that synapsis is followed by actual fusion? We may define an equation-division as *one that effects no reduction of valence*, a reduction-division as *one that reduces the valence to one-half*. This conforms exactly to the observed facts; and such a definition is, I think, equally consistent with complete disjunc-

tion or with a process of reconstruction subsequent to synapsis. I do not see how the analysis can be carried further without entering upon theoretical ground. Nevertheless, I do not hesitate to accept the probability that the reduction-division, as thus defined, involves a disjunction of chromatin-elements of some kind that are involved in the production of the unit-factors of heredity and that the Mendelian disjunction may here find its explanation (cf. De Vries, '03, Boveri, '04). It seems to me that the conclusions indicated by Boveri several years ago ('04) still remain the most probable; that is to say, that the degree of union may vary in different cases, involving sometimes no fusion (as is suggested by the history of the XY-pair), sometimes complete fusion, in other cases no more than a partial exchange of material. This point will be again touched upon in connection with Janssens's theory of the 'chiasmatype.'

The point that I wish here to emphasize is the validity of the conceptions of bivalence and the reduction-division, which have been more or less explicitly denied by a number of writers. I accept, of course, the conclusion of Haecker ('07), Bonnevie ('08, '11), Della Valle ('07), Popoff ('08) and others, that neither the heterotypical form of division nor an apparent tetrad-structure is necessarily diagnostic of bivalence or a reduction-division. Tetrad-like chromosomes have been repeatedly described in somatic divisions (see especially Della Valle, Popoff, cited above), and also in the univalent chromosomes of the second maturation-division a striking example of which is the 'd-chromosome' of *Nezara*, described in my seventh 'Study.' Bonnevie, especially, has demonstrated in *Nereis* and other forms the very close similarity of the somatic chromosomes (in the cleavage of the egg) to the heterotype-rings of the maturation-divisions. Her conclusion is:

Ich habe in meinen Objekten nicht nur für die Annahme einer Reduktionsteilung keinen einzigen Beweis finden können; meine Untersuchung hat auch ergeben, dass die früher in der heterotypischen Natur der ersten Reifungsteilung gesehene Stütze einer solchen Annahme sämtlich hinfällig sind . . . Die beiden Reifungsteilungen müssen daher, bis anderes bewiesen worden ist, als Aequationsteilungen aufgefasst werden ('08, p. 271, '11, p. 241).

Again, from the existence of tetrad-shaped chromosomes in certain somatic divisions of Amphibia, Della Valle ('07) concludes, "Tutte le precedenti formazioni e molto probabilmente, quando esistono, anche quelle della profase del primo fuso di maturazione, non hanno alcun rapporto con la riduzione cromatica, ma sono indice di una costituzione patologica dei cromosomi" (!). Both the foregoing passages, I think, like others of like import that might be cited, overshoot the mark. The significance of ring-shaped or tetrad-like chromosomes must be judged from a more critical standpoint than this. We must endeavor to discover their real meaning in each case by the study of their origin, their behavior in successive divisions, their morphological composition (whether simple or compound bodies), their relation to the conditions seen in other species, and any other facts that may throw light upon them. By way of illustration certain specific cases may be mentioned. A very interesting one is afforded by the *X*-chromosome of *Syromastes* (Gross, '04, Wilson, '09 a, '09 b), which is unaccompanied by a synaptic mate (*Y*-chromosome) yet forms a conspicuous 'tetrad' in the first spermatocyte-division. The reason here is that this 'chromosome' consists of two components, which appear as separate chromosomes in the spermatogonial groups but in the maturation-divisions are associated to form a double element which behaves precisely like the single *X*-chromosome of other species, and obviously corresponds to the latter because four such components are present in the diploid groups of the female (Wilson, '09 b). The '*d*-chromosome' of *Nezara* is a different but not less interesting case, always forming a conspicuous 'tetrad' in the *second* spermatocyte-division, but appearing as a single chromosome in the diploid groups. These two apparently contradictory cases are brought under the same point of view—especially when compared with the analogous relations described by Morgan ('09) in *Phylloxera*, and by Browne ('10) in *Notonecta*—if we assume that in each case the chromosome in question was originally a single body which in *Nezara* shows a slight tendency to separate into two components, in *Syromastes* has already done so (cf. Wilson, '11 a). Neither case offers

a real exception to the rule; for such 'tetrads' obviously differ entirely from the true tetrads of the maturation-processes, which represent synaptic pairs of the diploid groups. Perhaps an analogous case is that of *Cyclops* and *Diaptomus*, where Haecker ('95, '02) long since demonstrated a cross-bar or suture in each component of each bivalent; and since each of these component, is sooner or later longitudinally divided, double tetrads or 'ditetrads' are thus produced. The work of Braun ('09) and Matschek ('09, '10) confirms this but, like that of Haecker himself, proves that the cross suture is without significance for the divisions, since both the latter are longitudinal (cf. also Lerat, '05); while Krimmel ('10) has shown that in *Diaptomus* the transverse constriction or suture is also present in the univalent chromosomes of the diploid groups in *somatic* cells. Haecker's conclusion that the cross-suture represents the point of end to end synapsis is a quite unproved, and I think very improbable, assumption. In view of what is seen in *Syromastes*, *Notonecta*, *Phylloxera* and *Nezara*, it seems more likely that each univalent chromosome in these forms consists of two closely united components, of which the cross-suture is an expression. It seems quite possible that the number of chromosomes might change in these animals by complete separation of the two components in case of one or more of the chromosomes.

Keeping in view all such apparent exceptions, the fact remains that the heterotypic (or tetrad) form is a highly characteristic feature of the maturation prophase-bivalents, and that in this respect they show in general a marked contrast to the univalents, here and elsewhere. The meaning of this in case of the maturation-divisions is unmistakable; and the burden of proof may be left with those whose theoretical prepossession will not allow them to accept the natural explanation that here is manifest.

3. *The chromosomes and heredity*

That the chromatin-substance (more specifically that of the chromosomes) plays some definite rôle in determination has

received fresh support in recent years from several sources. Direct experimental evidence that is nearly if not quite demonstrative has been produced through the work of Boveri ('07) on multipolar mitosis, of Baltzer ('09, '10) on reciprocal crosses in sea-urchins, and of Herbst ('09) and Godlewski ('11) on the combined effects of artificial parthenogenesis and fertilization in hybridization. Indirect but very strong evidence has been given by the demonstration of a constant relation between particular chromosomes and sex and especially sex-limited characters (cf. Wilson, '11 a, b, Morgan, '11, Gulick, '11). This evidence in no manner precludes the view that the protoplasmic substances are also concerned in determination—indeed experimental embryology and cytology have produced very clear evidence that such is the case. The study of the nucleus, and especially of the chromosomes, offers however one of the most available paths of approach to a study of the activity of the germ-cells in determination, and for a detailed analysis of genetic problems in their cytological aspects.

It has been widely assumed that the Mendelian segregation depends upon a disjunction of chromatin-elements in the reduction-division, as was originally suggested by Guyer, Sutton, Boveri and Cannon. It has, however, become obvious from the experimental data that if this be so, these elements can not be individual chromosomes of fixed composition. This was first seen to follow from the fact, now apparently well determined in certain cases, that the number of independent allomorph-pairs may be greater than the number of chromosome-pairs. More recently the same result is demonstrated by the work of Bateson and Punnett ('11) on 'coupling' and 'repulsion' in certain plants, and by that of Morgan ('11 a) on sex-limited characters in *Drosophila*, which proves that an interchange of unit-factors must to some extent take place between homologous bearers of these factors in the germ-cells. The results of Morgan in particular nevertheless bring strong support to the view that the chromosomes are such bearers of unit-factors; for the whole series of phenomena determined in *Drosophila*, complicated as they seem, become at once intelligible under the assumption that certain factors necessary

for the production of the sex-limited characters are born by the *X*-chromosome; and without this assumption they are wholly mysterious.

Adopting this explanation, the history of certain of these sex-limited characters, as Morgan points out, demands the further assumption that in the female the factors for these characters may to some extent undergo an interchange between the two sex-chromosomes (here two *X*-chromosomes) while in the male such interchange does not take place. Such a difference between the sexes finds a perfectly simple explanation in cases where the *X*-chromosome of the male has no synaptic mate (*Y*-chromosome). When a *Y*-chromosome is present—as may be the case in *Drosophila* according to the cytological observations of Stevens ('08)—the problem becomes more complicated, but there are some facts that may be significant in this direction. It is well known that in the male the sex-chromosomes commonly retain a compact and rounded form (as 'chromosome-nucleoli') throughout the entire spermatogenesis, and in some cases (*Oncopeltus*) they conjugate while in this condition, and subsequently disjoin without ever having undergone fusion or even intimate union, such as is so characteristic of the autosomes during the maturation-process. Unfortunately the oögenesis is as yet but imperfectly known; but there is considerable evidence that in some forms the sex-chromosomes exhibit a quite different behavior from that characteristic of the spermatogenesis. My own observations ('06) seemed to show that in some of the Hemiptera the sex-chromosomes do not in the oöcyte retain a compact form at the period of synapsis, or in the early growth-period, and the later observations of Foot and Strobell ('07) show that the same is true for later stages of the germinal vesicle. The work of Morrill ('10) further shows that in the female the sex-chromosomes have already conjugated before the first oöcyte-division. These facts make it probable that in these forms the sex-chromosomes of the female show the same behavior in synapsis and reduction as the autosomes, and enter into the same intimate union that characterizes the latter. It is quite possible that in these facts we may find an

explanation of the difference of the sexes in respect to the interchange of sex-limited factors that is proved to take place by the experimental results.¹⁸

There is no a priori reason why such a process of free interchange between homologous chromosomes may not take place in the somatic or diploid nuclei. It is, however, far simpler to assume that it occurs during or subsequent to synapsis; for it is only at this period that the homologous chromosomes are intimately and regularly associated (cf. Boveri, Strasburger, '08, '09). It is these facts, taken together with the cytological evidence, that lead me to the conclusion already indicated that the synaptic union results in a reorganization of the chromosomes, and that the two moieties of the final prophase-chromosomes are probably not identical with the original conjugants. Janssens's theory of the chiasmatype ('09) gives the only simple mechanical explanation thus far offered as to how such an orderly exchange of materials may be effected; and his conception has recently been applied in an ingenious manner by Morgan ('11 b) to an explanation of both 'repulsion' and 'coupling' in heredity. The chiasmatype-theory has been criticized by Grégoire and by Bonnevie on the ground that a strepsinema stage occurs in the division of somatic nuclei as well as in the maturation-prophases, and that the original twisting together of the threads is in some cases followed by untwisting, without such a process of partial fusion and subsequent secondary splitting as is postulated by Janssens. There are two replies to this. One is that Janssens's theory is not an a priori construction but a conclusion based on a most accurate and detailed study of the actual conditions seen in the prophases of Amphibia which prove that such a process as he postulates must here take place. The other is that there is considerable evidence that a twisting together of the conjugating threads takes place in the process of synapsis, leading to a most intimate union of the

¹⁸ It would, however, be rash to generalize this statement at present, for the observations of Buchner on the female *Gryllus* ('09) and those of Winiwarter and Saintmont ('09) on the cat, have demonstrated a nucleolus-like body in the synaptic and later stages of the oocytes which may be the XX-bivalent, though this is unproved, and doubt is thrown upon the second of these cases by the recent work of Guthertz ('12).

two members of each pair, and followed by a longitudinal split. It would, no doubt, be premature definitely to accept this conclusion at present; but it seems worthy of more attentive consideration than it has yet received.

Turning to the more general aspects of these problems, in what sense may we be justified in speaking of the chromosomes as bearers of the 'determiners' or factors of determination? I have recently outlined in another place (Wilson, '12) my position in regard to this question, and will here only indicate its most essential features. The 'determiners' or 'factors' on which unit-characters depend need not be regarded as independent, self-propagating germs (pangens, biophores, or the like); it is sufficient for our purpose to think of them in a more vague way as only specific chemical entities of some kind. However they are conceived in this regard, it would be a fundamental error to regard them as 'bearers' of the *characters* that depend upon their presence or absence; for every character is produced as a reaction of the germ considered as a whole or unit-system. Characters are 'borne' (if the expression is permissible at all) by this system as a whole; and the 'unit-factors' or 'determiners' postulated by students of genetics need be considered only as specific, differential factors of ontogenetic reaction in a complex organic system. Many 'unit-characters' are known to depend upon a number of such unit-factors, in some cases probably upon a large number; and they may be definitely altered this way or that by varying the particular combinations of these factors. But any unit-factor produces its characteristic effect only in so far as it forms a part of a more general apparatus of ontogenetic reaction constituted directly or indirectly by the organism as a whole. In all this, a striking parallel exists between the physical basis of heredity and the complex molecular groups of organic substances such as the proteins. The relation of the 'determiners' to the qualities of the organism considered as a whole may be compared to that which exists between the protein 'Bausteine' and the qualities of the protein molecular group as a whole.

"Just as the qualities of a particular protein may be definitely altered by the addition, subtraction or the substitution one for another of parti-

cular side-chains or molecular 'Bausteine,' so the addition, subtraction or substitution of particular 'determiners' or 'factors' in the zygote calls forth specific responses that lead to the production of corresponding characters. The reasoning that applies to the first of these cases seems equally applicable to the second. No one, I suppose, would hold in the first case that the particular molecular groups or 'Bausteine' concerned in the change are 'bearers of' (i.e., are alone responsible for) the resulting new qualities. The qualities of any protein, as Kossel has recently urged, belong to the molecule as a whole, and are not to be regarded as the sum of the qualities of its constituent 'Bausteine.' Why should we regard in a different light the 'determiners' (chemical substances) concerned in the second case? They are, clearly, not to be regarded as 'bearers' or 'physical bases' of the characters which depend upon their presence or absence. They are, I repeat, only differential factors of ontogenetic reactions that belong to the germ considered as a whole or unit-system" (Wilson, '12).

Kossel ('12) makes the pregnant remark that every peculiarity of the species and every occurrence affecting the individual may be indicated by special combinations of protein 'Bausteine.' The facts lead us to seek for such compounds (substances) in the chromatin or the chromosomes. It can hardly be said that even a beginning has been made in the chemical investigation of the distribution of the chromatin-substances within the nucleus. Cytologically, however, a long series of the most significant facts have been made known in respect to their groupings and modes of distribution. Evidence steadily accumulates that these processes are perfectly ordered; and the fact is now more than ever evident that they run parallel to the factors of determination and heredity. There has been a disposition on the part of certain writers of late to minimize the definite order of the morphological transformations of the nucleus (cf. Fick, '07, Della Valle, '09); and these authors, among others, have undoubtedly helped to create an impression that these phenomena, particularly as regards the chromosomes, are too vague and fluctuating to afford trustworthy results on the side of cytological research. I believe this to be a backward step, though I am very ready to admit the service to accuracy of observation that may be rendered by so critical and sceptical a spirit. Plastic, and in some respects variable like other biological phenomena, these processes undoubtedly are; but the more one studies them in detail the stronger grows the con-

viction of an exact order that underlies their superficial fluctuations, and one of which the main outlines are steadily becoming clearer.

It appears to me that many of the recent cytological advances support the view that the true key to this order was found by Flemming when he chose the word 'mitosis' ('82), and by Roux in his attempt to find the essential meaning of the mitotic process ('83). This is well illustrated by the pre-synaptic phenomena that have here been considered, and by the increasing body of observations that emphasize the importance of the mitotic transformation of the chromatin-substance. It is difficult to see what meaning such processes can have if they do not involve a linear alignment of different elements (substances) which are thus brought into a particular disposition for ensuing processes of division (Roux) or of paired association (Strasburger).¹⁹ The practical utility of such a conception for the analysis of genetic problems has already become apparent. It is still only an hypothesis, but one which we may hope sooner or later to see subjected to definite experimental test.

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¹⁹ This point has been forcibly urged by Strasburger (see '08, pp. 563-568; '09, pp. 95-97).

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PLATE 1²⁰

EXPLANATION OF FIGURES

All of the figures are from *Oncopeltus fasciatus* excepting 7 and 8, which are from *Lygaeus bicrucis*. Enlargement 2250 diameters.

1-3 Spermatogonial metaphases. *Oncopeltus*.

4-5 Metaphases from ovarian cells.

6 Spermatogonial metaphase. *Lygaeus*.

7 Metaphase of first spermatocyte-division. *Lygaeus*.

8-13 Metaphases of first spermatocyte-division in polar view. *Oncopeltus*.

14-17 Metaphases of same in lateral view, showing the sex-chromosomes near the center.

18 Early anaphase of same division, showing all the chromosomes. The two at the right and the one at the left have been drawn displaced so as not to confuse the central group.

19 Later anaphase, showing approach of the sex-chromosomes.

20 Slightly later stage, showing the sex-chromosomes in contact at each end of the spindle.

21 Slightly earlier anaphase, from a smear-preparation, showing all the chromosomes. The sex-chromosomes already in conjugation at each pole.

²⁰ All of the figures of plates 1 to 7 have been drawn as far as possible with the camera lucida, though of course at so great an enlargement it has been necessary to finish much of the finer detail freehand. Many of them are the work of Miss Mabel L. Hedge, to whose skill and painstaking accuracy especial acknowledgment is due. In many cases (as indicated in brackets) the same objects are shown by photographs on plates 8 to 9. It should be added that some of the finer details appear less clearly in the reproductions of these photographs than in the original negatives.

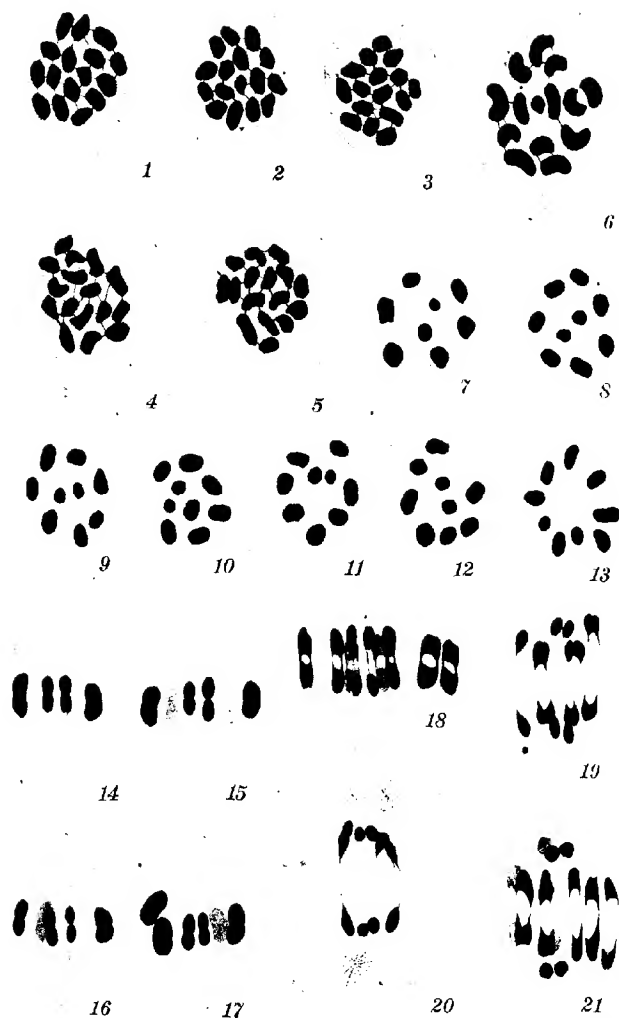


PLATE 2

EXPLANATION OF FIGURES

Onco peltus, excepting 28, 29, 45, 46, which are from *Lygaeus*. Enlargement 2250 diameters.

22-23 Final anaphases of the first division. The sex-chromosomes not visible in these views.

24-25 Polar views of two daughter-chromosome plates from the same stage as the foregoing (from different spindles) showing the *XY*-bivalent near the center (no. 712).

26-27 Similar views of sister-groups from the same spindle (no. 760).

28-29 Sister-groups from the same spindle. *Lygaeus*.

30-32 Interkineses, showing the chromosome-groups, the first in side-views, the others in face-view showing all the chromosomes (no. 760).

33 Prophase of second division.

34-35 Second division metaphase in polar view.

36-44 The same in side-view, the sex-chromosomes seen separating in several of the figures (36-41, equal type, no. 712; 42-44, unequal type, no. 760).

45-46 (photo. 6) Second division metaphases of *Lygaeus*, lying side by side in the same section, one in polar view, one from the side.

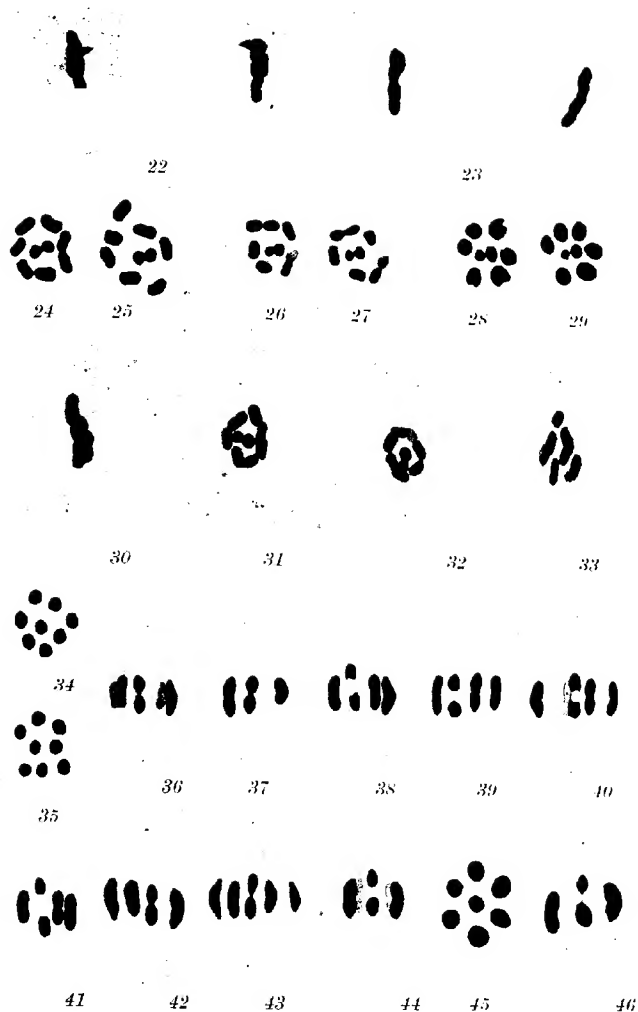


PLATE 3

EXPLANATION OF FIGURES

Oncopeltus, from sections excepting 65, which is from a smear-preparation. Enlargement 2250 diameters.

47-48 Spermatogonial telophases. It is uncertain whether this is the last division or an earlier one.

49 Later spermatogonial telophase. Probably Stage *a*.

50-51 Stage *b*, showing the massive chromatic bodies, the sex-chromosomes readily distinguishable.

52-55 Stages *b-c*, showing the process of unravelling.

56-59 Stage *d*. Leptotene-nuclei.

60 Transition to the synizesis. Synaptic period.

61 Stage *e*. Synizesis, from a very clear specimen.

62 Transition to the following stage.

63 Stage *f*. Pachytene-nucleus. The threads apparently undivided.

64 Stage *f*. Diplotene-nucleus.

65 (photo. 10). Stage *f*. Diplotene-nucleus, from a smear-preparation.

66-67 Stage *g*. The confused stage, showing plasmasome and both chromosome-nucleoli (sex-chromosomes). Plasmasome at its maximum size.

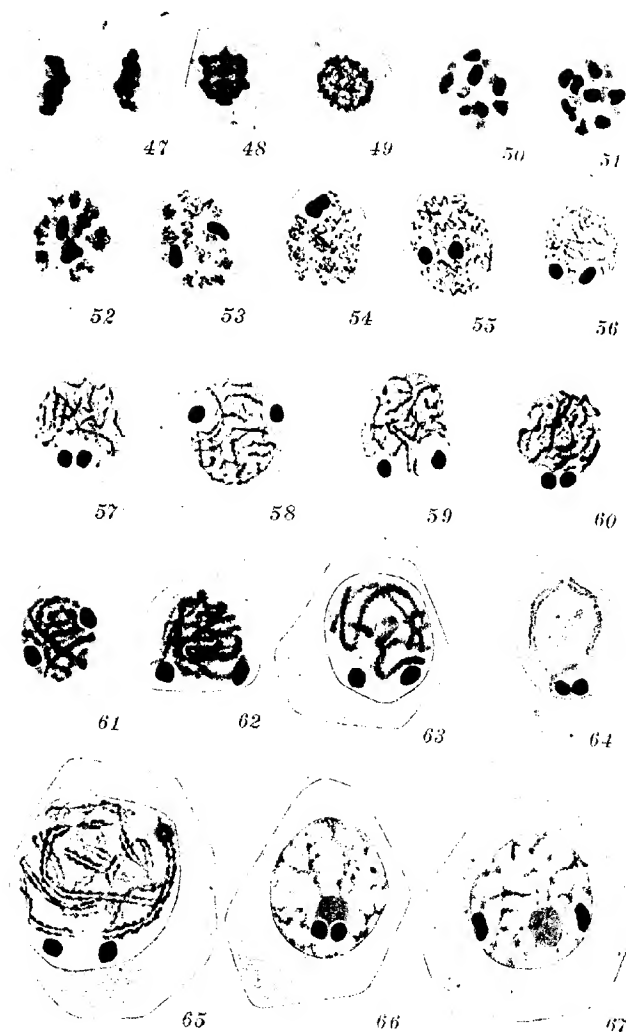


PLATE 4

EXPLANATION OF FIGURES

Lygacus bicrucis (68-73, 83, 84), *Largus cinctus* (74-82), *Anax junius* (85-87), *Achurum* (88-92). Enlargement 2250, excepting the figures of *Achurum*, which are enlarged 1500 diameters.

68-70 Stage *a*. Earlier and later final spermatogonial telophases, from the same cyst. *Lygaeus*.

71-72 Stage *b*. *Lygaeus*.

73 *a*-73 *b* Stage *d*. Leptotene-nuclei. *Lygaeus*.

74-75 Spermatogonial telophases, from the same cyst. *Largus*.

76-78 Stage *c*. *Largus*.

79-80 Stage *d*. Leptotene-nuclei. *Largus*.

81-82 Stage *f*. Diplotene-nuclei. *Largus*.

83-84 The same. *Lygaeus*.

85-87 Stages *b*-*c*. *Anax*.

88-92 Stages *b*-*c*. *Achurum*.

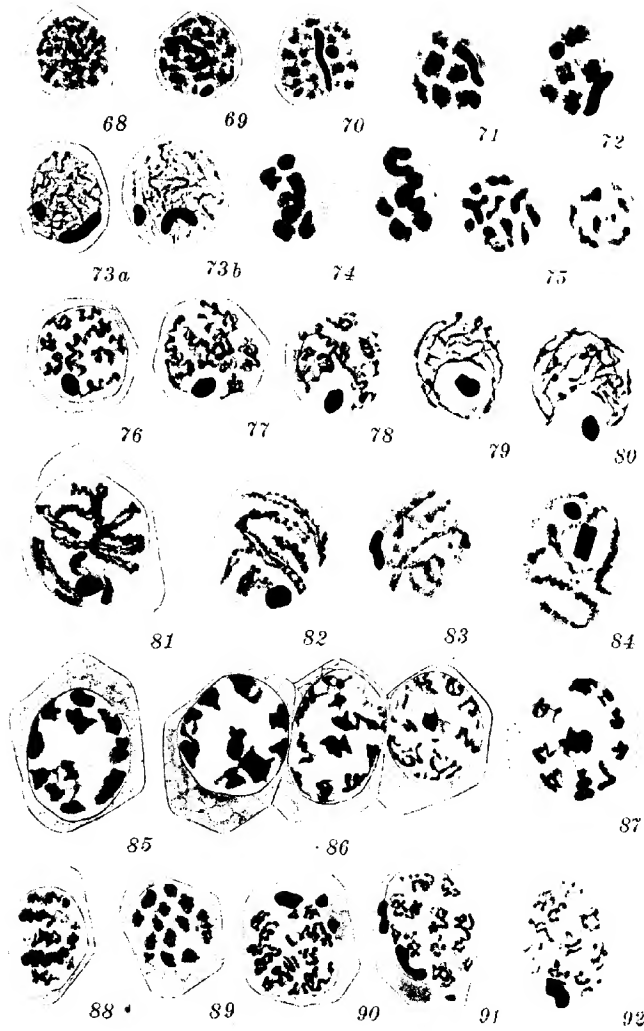


PLATE 5

EXPLANATION OF FIGURES .

Phrynotettix (93-96), Lygaeus (97-100), Largus (101-104), Oncopeltus (105-108). From sections, excepting 107, 108, which are from smear-preparations. The figures of Phrynotettix enlarged 1500 diameters, the others 2250 diameters.

93-95 Spermatogonial prophases of Phrynotettix. Fig. 93 is an early stage, showing massive polarized bodies. The other figures show the uncoiling of the spireme-threads from these bodies, 94 in side view (photo. 31), 95 as viewed from the pole (photo. 30). Fig. 96 shows two successive stages lying side by side in the same cyst (photo. 32).

97 The confused stage (Stage *g*) in Lygaeus.

98-99 Early prophases (Stage *h*) from Lygaeus.

100 *a-h* Isolated chromosome-nucleoli, from Stages *f* and *g* in Lygaeus, showing various forms of the sex-chromosomes assumed during the growth-period.

101-103 Largus cinctus. Nuclei transitional from Stage *f* (diplotene) to Stage *g* (confused period).

104 Nucleus of the confused period. Largus cinctus.

105-106 Early prophase-nuclei, Oncopeltus (early Stage *h*).

107 (photo. 17) Slightly later prophase-nucleus of Oncopeltus, showing early bivalents.

108 (photo. 18) Later prophase of the same, early Stage *i*.

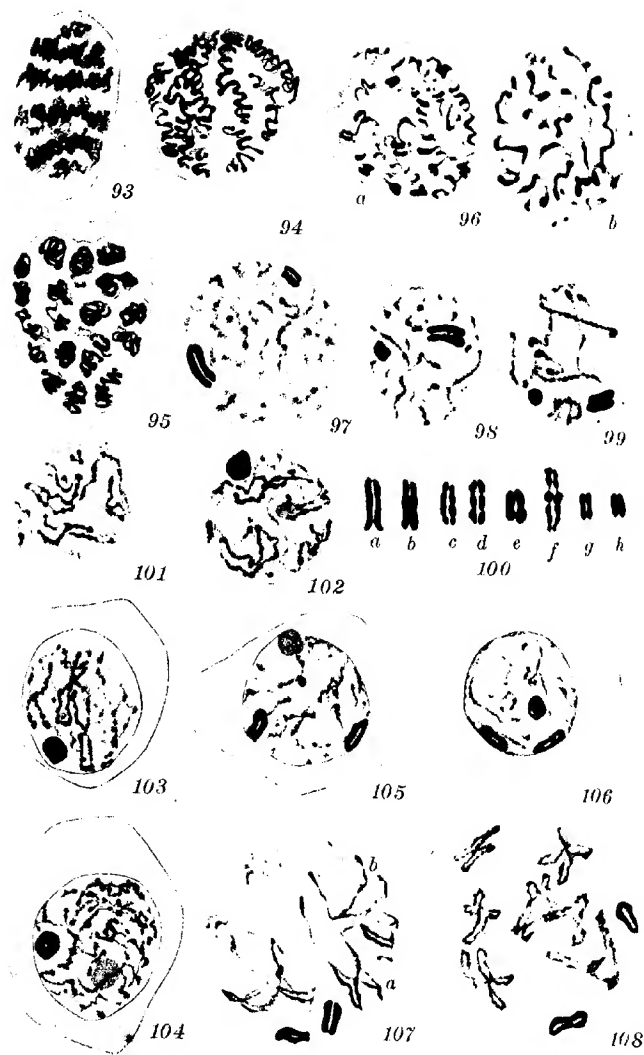


PLATE 6

EXPLANATION OF FIGURES

From smear-preparations of *Oncopeltus* (109-114) and *Protenor* (115-120); Enlargement 2250 diameters. (*X* designates the *X*-chromosome, *B* the large bivalent in *Protenor*, *m*, *m*, the *m*-chromosomes in the latter form.

109-114 Middle and late prophases of the first spermatocyte-division, showing various forms of the bivalents during their condensation. In the earlier figures the *X*- and *Y*-chromosomes are short, longitudinally split rods (109-111); in the later ones they are shortening to a dumb-bell form (112-114). Two of the same nuclei are shown in photos. 20, 21.

115-117 Early prophases (Stage *h*), the bivalents just emerging from the confused stage. The *m*-chromosomes are but vaguely distinguishable.

118-119 Late Stage *h*, showing all the chromosomes, the bivalents still much diffused.

120 (photo. 40) Nucleus from Stage *i*.

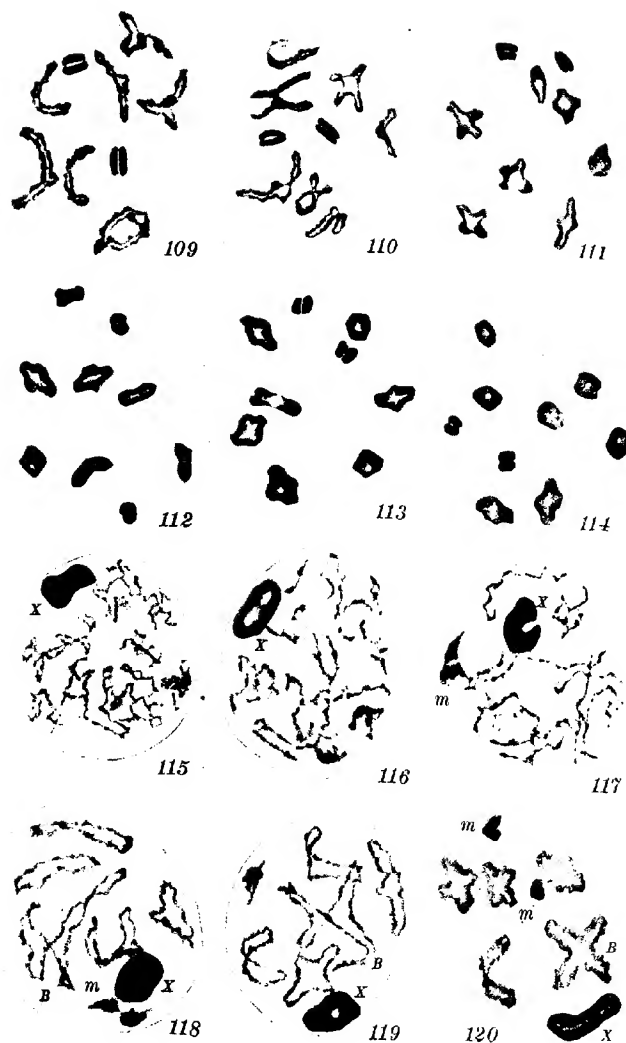


PLATE 7

EXPLANATION OF FIGURES

From smear-preparations of *Protenor*; 2250 diameters; (lettering as in the preceding plate).

121-127 Middle prophases (Stage *i*) showing all the chromosomes. The *m*-chromosomes, now condensed, are separate in all but 124. In 126 the large bivalent is a straight rod; in 127 it is such a rod bent at the middle point to form a V (here seen edgewise so as not to show the longitudinal cleft. Some of the same nuclei are shown in photos. 41, 44, 47.

128 (photo. 43) Abnormal nucleus in which the large bivalent is represented by a pair of separate univalents (*B, B*) that have failed to unite in synopsis.

129-131 Late prophases (Stage *j*). In 131 the chromosomes are ready to enter the metaphase-plate; (fig. 131 also in photo. 51).

132-134 Early metaphases. In 132 one of the small bivalents and the *m*-chromosomes appear abnormally large, owing to flattening. In 134 the ring tetrad to the left has been slightly displaced in order to show it more clearly.



Heller, Del.

PLATE 8

EXPLANATION OF FIGURES

From photographs by the author. Enlargement a little less than 1250 diameters. *Oncopeltus* (1-5, 7-11, 16-24), *Lygaeus bicrucis* (6, 12-15, 25), *Largus cinctus* (26, 27). Many figures of the same preparations, from drawings, are reproduced in the preceding plates, as indicated in brackets. Photos. 1-15, 26, 27, from sections, the others from smear-preparations.

- 1, 2 Spermatogonial metaphases. *Oncopeltus*.
- 3 First division metaphase.
- 4 Second division metaphase.
- 5 First division metaphase in side view, showing the sex-chromosomes in the center.
- 6 (Figs. 45, 46) Second division metaphases, one in polar view, one in side view, showing the initial separation of *X* and *Y*. *Lygaeus bicrucis*.
- 7-8 Stage *d*. Leptotene-nuclei of *Oncopeltus*.
- 9 Pachytene-nuclei, just emerging from the synzesis. *Oncopeltus*.
- 10 (Fig. 65). Stage *f*. Early diplotene-nucleus. From a smear.
- 11 Stage *g*. Confused stage, showing plasmasome and both sex-chromosomes.
- 12 Stage *e*. Synzesis, *Lygaeus*, from much extracted preparation, showing the *X*- and *Y*-chromosomes united.
- 13 Above, the *X*- and *Y*-chromosomes of *Lygaeus* in Stage *f*, attached in one case end to end, in the other side by side. Below, the same from early Stage *g*, showing also the plasmasome.
- 14-15 Nuclei of Stage *g*, *Lygaeus*, showing the longitudinally divided *X*-chromosome, and (at the left) the plasmasome.
- 16 Nucleus of the confused period (Stage *g*). *Oncopeltus*.
- 17-24 Early, middle and late prophase (Stages *k-j*) from smear-preparations of *Oncopeltus*. Photo. 17 (fig. 107), 18 (fig. 108), 20 (fig. 109), 21 (fig. 111). The sex-chromosomes distinguishable in each case.
- 25 Late prophase-nucleus of *Lygaeus* (Stage *i-j*), the *X*- and *Y*-chromosomes readily distinguishable above towards the left.
- 26-27 Stage *b-c*, in *Largus cinctus*. The uncoiling of spiral leptotene-threads is clearly visible in the negative of photo. 27.

STUDIES ON CHROMOSOMES. VIII.

EDMUND B. WILSON



PLATE 9

EXPLANATION OF FIGURES

From photographs by the author. Enlargement as in the preceding plate. Photos. 28-38 from sections (28-32 from McClung's preparations), 39-51 from smear-preparations. Photo. 28, *Achurum*, 29-32 *Phrynotettix*, 33, 34 *Largus cinctus*, 35-51 *Protenor belfragei*.

28 Stage *c* in *Achurum*. The unravelling threads clearly shown in the negative.

29 Early spermatogonial prophase of *Phrynotettix*, showing the massive chromatin-bodies just before the spiral thread is evident.

30 (Fig. 95). At the left, polar view of the coiled threads during the early uncoiling, spermatogonial prophase.

31 (Fig. 94). The same stage (from a nucleus immediately adjoining in the same section) seen in side-view.

32 (Fig. 96). Two adjoining nuclei, showing at the left an earlier, and at the right a later stage of the uncoiling of the spireme-threads. The drawings of these and the preceding photo. show threads at other levels as well.

33 Spermatogonial metaphase of *Largus cinctus*, 11 chromosomes, including one large pair. The *X*-chromosome is one of the smaller ones, and can not be distinguished by the eye.

34 Metaphase of diploid group of the female of the same species, 12 chromosomes.

35 (Fig. 1 *e*, Wilson, '06). Spermatogonial metaphase of *Protenor*; 13 chromosomes.

36 The same. In both these photos. the large *X*-chromosome and the large pair of autosomes are readily distinguishable.

37-38 Diploid chromosome-groups of the female *Protenor*, showing the *X*-pair and the large pair of autosomes; 14 chromosomes.

39 *Protenor*. Above, a nucleus of the confused stage (*g*) showing the elongate *X*-chromosome. Below are three final anaphases of the second division, showing the passage of the undivided *X*-chromosome to one pole.

40-51 Prophase-nuclei of *Protenor*. Photo. 40 (fig. 120), 41 (fig. 122) 43 (fig. 123), 44 (fig. 121), 46 (fig. 129), 47 (fig. 127), 51 (fig. 131).

STUDIES ON CHROMOSOMES. VII.
 TETRAPOID, WILSON

PLATE I



WOUND CLOSURE AND POLARITY IN THE TENTACLE OF METRIDIDIUM MARGINATUM

WAYLAND M. CHESTER

EIGHT FIGURES

The following study was begun by S. Stillman Berry and the author at the Zoological Laboratory of Harvard University in 1909, and continued by the author at Woods Hole during the summer of 1911. Mr. Berry should be particularly credited with the initiation of the work on polarity, and I have made use of his notes and compared his results with mine in the progress of the study. I am indebted to Dr. Herbert W. Rand, under whose direction the study was made, for many helpful suggestions. Thanks are due Dr. F. B. Sumner, as Director of the Laboratory of the Bureau of Fisheries at Woods Hole, for facilities for work during the summer of 1911.

The only previous study of the wound reactions of the tentacles of actinians is that of Rand ('09). He described and analyzed the activities involved in the closing of wounded tentacles and discussed the phenomena of polarity in the large southern forms, *Condylactis* and *Aiptasia*, found at Bermuda.

WOUND CLOSURE

The present observations were made upon medium sized individuals of *Metridium marginatum*. When expanded, the column carries a disk that is of larger diameter than the column and has an outer zone, near its convoluted margin, of many rows of tentacles, which are conical. Those of the inner row are somewhat larger than the others. The animals used for experiment had a column 25 to 35 mm. high and 25 to 30 mm. in diameter. The tentacles of the inner row were 10 to 15 mm. in length with a

diameter of 1 mm. at the base and 0.1 mm. at the tip. Except where otherwise stated the results are with tentacles of approximately this size.

A tentacle of such an animal, when fully expanded, was cut off with sharp scissors about midway of its length. The immediate result was the collapse of the tentacle stump, owing to the pressure of the liquid within the tentacle being released; and this was followed by its contraction to the disk. The collapse and contraction of the tentacle were accompanied, however, by the bending of the adjacent tentacles toward it, and the contraction of the disk at that point, so that a marked indentation and infolding occurred there. Then the disk as a whole rolled in and the column sphincter closed. The tentacle stump was hidden by the adjacent tentacles and by the inrolling of the disk for nearly two minutes. At the end of that time the disk had regained its expanded condition and the tentacles adjacent to the stump were expanding. The tentacle stump was also expanding and, within five minutes, its distal end meanwhile having been closed, was extended almost as much as before the cut was made. The closure of the tentacle had resulted in a rounded end, which carried a short and constricted nipple-like protrusion (fig. 1). Within half an hour the protrusion was, if anything, more constricted and the tentacle appeared more inflated than its neighbors, or than it was before the cut was made. The marked contrast to the adjacent tentacles is in part due to a change of shape, for it is now cylindrical from its base to the rounded end, whereas, before it was cut, it was conical. Figure 1 shows the difference of shape between the cut and uncut tentacle one hour after the cut was made.

Light brown animals were found to be better for observation than the darker specimens. The endoderm of their tentacles, or a part of it, is black. At the constricted end of the cut tentacle, this shows as a black plug in the axis (fig. 1). The exterior of the constriction is white and opaque, but where the tentacle resumes its normal diameter this white color grades into the usual pale brown. The black of the endoderm as seen in optical section is shown in figures 1, 2, 4, 5 and 6 by stippling.

In the experiment described the tentacle was cut midway of its length. The reactions in more than fifty experiments with tentacles cut in this region varied somewhat, but the results, as regards the closure and the resulting form of the distal cut end, were in all cases like those in the experiment described. The tentacle always collapsed after the cut and contracted toward its base. There were, however, variations in the amount of activity of the disk and the adjacent tentacles. Sometimes the disk

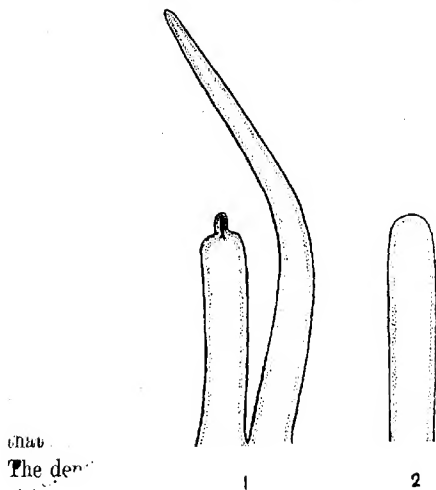


Fig. 1 A normal tentacle and the stump of a second tentacle one hour after removal of its distal portion. $\times 5$.

Fig. 2 Stump of second tentacle twenty-four hours later. $\times 5$.

showed no great movement. The tentacle stump collapsed and the adjacent tentacles bent over it. Sometimes, in sluggish animals, the activity was limited to the collapse and contraction of the tentacle that was cut. Consequently the time of the first appearance of the stump after cutting and contraction varied; but full expansion always showed the same result. Usually the cut tentacle could be seen within five minutes, and was fully expanded within ten minutes.

When the cut was carefully made near the tip, not so often did all parts of this series of results occur. If very near the tip, the tentacle alone reacted, scarcely contracting, the tip closing immediately before collapse could occur. If the cut was between the middle and the base of the tentacle, the series of reactions involving the stump, the adjacent tentacles and the disk almost always occurred, as described above, and the tentacle was hidden for a longer period. In either case, however, the closed end resembled that of the tentacle cut midway of its length, though it reached its normal expansion very quickly in a cut at the tip, and very slowly in a cut at the basal end. In the latter case five to ten minutes usually elapsed, and it was sometimes hidden for twenty to thirty minutes.

The phenomena of closure and the resulting form were not greatly different from those found in *Condylactis* and *Aiptasia* (Rand, '09, pp. 195-201). The polyps of the *Metridium* used had tentacles that, while tapering as in *Condylactis*, were very much smaller (one-ninth or one-tenth the length). The basal diameter was also less than that of the cylindrical tentacles of *Aiptasia*. The resultant nipple was short, with proportions, relative to those of the tentacle, not greatly different from those of the nipple in *Condylactis*; but it was not as long, relatively, as that of *Aiptasia*. The thin black endodermic layer of the otherwise light brown tentacle of the *Metridium* polyps became very conspicuous in the nipple as an axial plug, while the tentacle wall showed a ~~hazy~~ ^{hazy} for it as in *Condylactis*, from the dense opacity of the nipple to the translucent brown of the tentacle.

Rand ('09, p. 198-201) found that in the southern forms three successive phenomena are involved in the closing of the wounded tentacle. These are, first, a slight and immediate inrolling of the cut edge; secondly, the formation of a muscular sphincter or nipple; and, thirdly, the gradual replacement of the nipple by a closed rounded end.

In a number of my experiments the wounded tentacle collapsed and contracted while the adjacent tentacles did not hide it. In these cases, the activities could be watched from the time of wounding. When the cut was first made, the edges rolled slightly

inward, so that the diameter of the opening was perceptibly smaller than that of the tentacle. The inrolling must be caused by an inequality of tension which is normally present in the cell layers of the wall.

As the expanding tentacle became filled with the body fluid, the circular as well as the longitudinal muscles relaxed, except for the space of one millimeter at the cut end. Consequently the tentacle stump, except at its extreme tip, was larger in diameter in the distal part of its length than before it was cut. The closure had been effected, following the initial inrolling, by the formation of a prominent sphincter, presumably due to the action of the circular muscles of a narrow zone at the cut end. The evidence of an existing sphincter began with the expansion of the tentacle, and this sphincter gradually but quickly contracted as the body fluid flowed into the tentacle. With the formation of a nipple, the color of the region changed, the dark endoderm becoming darker as an axial plug, and the ectoderm becoming more opaque. The color of the nipple graded into the light brown of the tentacle's rounded tip.

That the nipple is muscular in character in *Condylactis* and *Aiptasia* is shown, according to Rand ('09, p. 210), by these facts: The form of the cut tentacle is that of an inflated structure closed by a strong contraction at its tip. The color of the nipple is like that of tentacle tissue when under strong muscular contraction. The denser color of the closed end shades into the normal color at the place where muscular relaxation is found. The quickness of the sphincter-like action when the tentacle expands, indicates that the closure cannot be due to growth of tissues or to amoeboid migration of cells. The stump upon stimulation sometimes reacts by contracting, whereupon the nipple disappears for a time but reappears as the stump expands. Further, the tentacle end, which re-opens when the nipple disappears, shows an orifice whose size varies inversely as the length of time after the cutting of the tentacle.

These conditions were found in *Metridium* also. The color, form, and the quickness of the formation of the nipple have been described. The re-opening of a cut end upon contraction of the

stump was seen in almost all the animals observed. If the tentacle were stimulated to contract during the first hour after cutting, the nipple disappeared; but it quickly formed again when the tentacle expanded.

It was found possible by means of chemical reagents to eliminate the muscular activity at the time when ordinarily the nipple formed. The *Metridium* was stupefied by chloretone, under the influence of which it was kept for a greater or less length of time. In some experiments magnesium sulphate instead of chloretone was used, but, while the inhibition of the muscles was successful, the secretion of mucus was greater and the animal was not so easily controlled. A few chloretone crystals were placed on the surface of the water. When the tentacles ceased to respond to touch, some of them were severed. The initial in-rolling of the cut edges occurred, but there was no contraction of the tentacle and no formation of a nipple. In all cases, eight hours after a tentacle was cut, the opening at the distal end of the stump was still present and there had been neither formation of a nipple nor change of color such as accompanies contraction of the tissues. The opening very gradually decreased in size during this time, but it was a slow radial closing with no suggestion of the nipple-like form. When the cut was midway of the tentacle, complete closure occurred in from ten to twelve hours. The animal was left in the chloretone during the closing of the wound and then returned to normal sea water. In the twelve experiments in which this was done, structural closure was effected without the formation of a nipple. In other experiments, when the animal was returned to normal sea-water at some stage before complete closure of the cut end, a nipple was formed upon renewal of muscular activity.

The third phase in wound reaction consisted in the structural closure of the cut end, accompanied by the relaxation of the nipple sphincter. Twelve to twenty-four hours after the nipple was first formed, the end of the tentacle appeared inflated, and was hemispherical in form (fig. 2). During that time the nipple had gradually disappeared, but, for a few hours after the end became rounded, a white spot could be seen at the point where the nipple

had been. On the second day the tentacle was inflated, had a rounded end, and the color was alike in all parts. Not only was this permanent structural closure not effected by muscle action, but the muscular contraction of the nipple was diminished as it proceeded. It was a process of radial in-closing so gradual as not to be perceived save by the observation that, at successive intervals, the nipple was shorter, and the opening, when the stump was stimulated to contract, was smaller.

Some study was made of this closure of the cut edges after the muscle action was eliminated (fig. 3). In cut tentacles subjected to chloretone there is a movement of the tissues of the tip radially

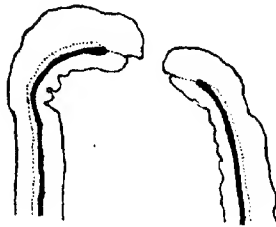


Fig. 3 Camera sketch of longitudinal section of the distal cut end of a tentacle stump after six and one-half hours in chloretone solution. The ectoderm and entoderm have proceeded beyond the mesoglea (black). $\times 60$.

across the end of the tentacle, the pressure of fluid within holding the end in rounded form as it closes. The process is, in effect, somewhat like the initial inrolling; but that is abrupt while this is gradual and slow. Other causes probably underlie the first inrolling of the edges. In the final closure, the ectoderm moves in advance of the other tissues, retaining its normal thickness for some distance from the edge, though the cells become arranged obliquely to the surface of the layer.

The appearance of a longitudinal section of a closed tentacle is shown in figure 8, which represents the closed end of a tentacle fragment which was not subjected to chloretone. Near the region of union of the ectoderm of the opposite sides of the orifice, the cells differ in shape from the adjacent normal cells, being much shorter so that the layer is thinner at the region where the meeting occurs.

The endoderm lags behind the ectoderm in this closing process more so in some tentacles than in others. Its region of advance is marked by a thinner layer of cells. The mesoglea is bent in by the inrolling process (fig. 7), but in the immediate region of the closure no mesoglea is found in any sections. In the region where ectoderm and endoderm are advancing beyond the mesoglea the muscle and ganglion cells do not appear, but the nettle cells and gland cells are abundant, except within a very limited region at the immediate edge of the advancing layers, where the cells are shorter and irregular in shape and the cell walls are not prominent. Here nettle cells and gland cells do not occur.

The unequal tension of the layers seems to be a large factor in the approximation of the cut edges; but other causes also work to carry the ectoderm and endoderm centripetally across the cut end. To a large extent this is done without great change in the layers. Only very near the advancing edge are conditions seen which more nearly suggest a migration of individual cells; but in this migration the more specialized cells take little or no part.

In the normal closure of the distal end of an attached tentacle stump, the cut edges are approximated largely by the action of the sphincter, and closure undoubtedly occurs without great migration of individual cells or changes in the relative position of cell layers. Sections of such a tentacle stump, the animal having been kept in normal sea water twenty-four hours after the tentacle was cut, show, in contrast with the conditions seen in the closure of a detached fragment (fig. 8), that the cell layers in the region of closure are not shallow and irregular, and that both mesoglea and muscle cells are normally distributed.

When the tentacle was severed obliquely, the closure took place in the same way as when the cut was directly across it. If the oblique cut extended completely through the tentacle, or nearly so the result was the formation of a nipple like that described, except that its distal surface was oblique to its axis. A sphincter formed even if the cut included no more than half the transverse section of the tentacle. When the cut extended less than half way across there was a bend of the tentacle at the place of injury and a slight contraction of the muscles near the cut.

POLARITY

The results of tactile stimulation showed in the *Metridium* the very significant physiological polarity that was found by Rand ('09, p: 223) in the southern actinians. This polarity consists in differences in the reactions on the proximal and distal sides of the point of stimulation. My results, however, differ from those of Dr. Rand in the details of the reactions. When a tentacle is gently touched with a glass rod or needle point, there is a slight thickening of a narrow zone proximal to the point of contact. This is evident by the barely perceptible enlargement of the girth of the zone and by the lighter color as the wall becomes opaque. In sluggish animals, viewed under a low power lens, the contraction is evident, not only by the change of color, but by wrinkling of the contracted band. This band is undoubtedly due to a contraction of longitudinal muscle fibers, but the contraction takes place only on the proximal side of the point of contact. In a more vigorous animal, or with a stronger stimulation of the tentacle of a sluggish one, the touch of the needle point produces an evident bending of the tentacle at the place touched and toward the point of contact; and this may be immediately followed by the swaying of the tentacle. The swaying, however, is produced by contractions that are entirely proximal to the point of contact, and the distal part of the tentacle is thereby bent stiffly first one way and then another. The bending of the tentacle toward the point of contact is not always followed by the swaying of the tentacle. This latter part of the reaction to touch is similar to the reaction to the approximation of food, in which the tentacle moves to and fro. It differs in the localization of the muscular contraction. In the touch reaction, the contraction is entirely proximal to the point of contact; in the feeding reaction, there may be contraction in the whole length of the tentacle. In the reaction to touch, the distal part is moved by the bending of the proximal; in the feeding reaction the whole tentacle waves. If the tentacle is touched when the distal part is curved or bent, this part may appear to wave. But it is simply carried through the water by the movement of the proximal part of the tentacle. A general

stimulus, such as food or the shaking of a tentacle, causes a response along its whole length. But when the stimulus was surely local, I did not see any contraction of the part distal to the point touched; and even when the tentacle was vigorously prodded, the distal reactions surely lagged behind those of the proximal part of the tentacle.

A variation in the response sometimes occurs. Instead of a bending of the tentacle at the point of contact toward the stimulus the opposite result may occur,—the tentacle bends at the point of contact away from the stimulus. We may call the bending toward the stimulated side, positive; the reverse movement, negative. Mr. Berry's notes show that he also saw both the positive and negative reactions, and the conditions under which they occur. The nature of the reaction depends upon the general physiological conditions of the animal. It exhibits at least two distinctly different conditions, and these were often tested. When the tentacles refused food, many, if not all of them, responded negatively to touch stimulation. This condition was quite likely to be preceded by a state in which some of the tentacles reacted negatively and others positively, or one in which a tentacle responded first one way, and then with a succeeding stimulus, the other way. At such time, the animal was usually sluggish, and the reaction was quite often not accompanied by the swaying nor by any contraction other than that of the narrow zone which first appears when a tentacle is touched. Also after the polyp had been taken from its attachment, and before it had become re-attached, or for a short time afterwards, the negative reaction occurred. After it had been attached for a day, the positive reactions appeared.

Particularly significant are the facts, first, that there are two different actions, and, secondly, that in both the positive and negative bending, the muscular activity is proximal to the point of stimulation.

A more general contraction of the basal part of the tentacle may follow the local bending and swaying, or it may occur at the same time as the local contractions. The proximal part of the contracted tentacle is thereby much shortened and its walls are thickened. In some instances the base expanded to a bulb-like shape, the

contraction of the longitudinal fibers being accompanied by the relaxation of the circular ones. Emphasis should be given to the fact that the three features of the reaction to touch, namely, the local zone of shortening, the bending at the point of stimulus, and the general longitudinal contraction, all involve only that part of the tentacle lying proximal to the point of stimulation.

Parker ('96) observed that in *Metridium* the cilia of the ectoderm of the tentacle make a current in the water toward the tentacle tip, and that, by means of the cilia, excised tentacles move for a long time with their basal ends directed forward. Tentacle fragments were kept alive in my experiments sometimes for eight days, and in one of Mr. Berry's experiments for a longer period. The cilia moved them during this time in the direction of their basal ends. Dr. Rand found that in *Condylactis* and *Aiptasia* also the cilia of the tentacle beat toward the tips of the tentacles.

One series of our experiments dealt with the behavior of the cut ends of the excised part of the tentacle. When the expanded tentacle was severed, if carefully done, the part cut off contracted in length but slightly, as compared with the stump, but an excised part never regained its full expansion after it contracted. After excision the tentacle was cut into two or more pieces. The level of the cuts varied in different experiments. The following results are from an experiment in which, after the tentacle was severed from the animal, two cuts were made, dividing the excised tentacle into three fragments (fig. 4, *a, b, c*). The tip (*c*) was first cut off. It scarcely contracted, while the proximal fragment contracted greatly. When, after a few minutes, the proximal fragment had expanded, the cut between *b* and *a* was made. The distal fragment resulting from this operation scarcely contracted, but the proximal part contracted strongly. Under low magnification all fragments showed transverse wrinkles; these are not shown in the figures.

The activity of the distal ends of *a* and *b* (fig. 4) should be compared with that of the distal end of the attached stump from which the tentacle had been cut (as in fig. 1). At the distal cut end of a fragment the abrupt initial inrolling of the edge was followed by a process of quite different character. The opening became smaller

and in some cases was closed within a half hour, though in many experiments it took five or six hours. Viewed by transmitted light under low magnification, the dark endoderm at the cut end appeared contracted for a distance that corresponded to the length of the nipple on an attached stump. The ectoderm of the same region was white and opaque, the opacity diminishing peripherally as in the stump end. On the second day the distal ends of the fragments were closed and rounded (fig. 5, *a* and *b*). The activity at the distal ends of excised pieces is therefore much the same as that in the end of a stump. At the distal end of an excised fragment there is a muscular contraction corresponding to the temporary muscular closure in the stump. But the sphincter in the fragment does not close so tightly and a nipple is not often found. A structural closure occurs similarly in the stump and the distal tips of the fragments (compare figs. 2 and 5).

In order to compare further the behavior of distal cut ends when the tentacle is attached and when it is free, I performed the following experiment a number of times with uniform results. A piece of tentacle was cut off and when the stump had formed its nipple, the end with the nipple was cut off and allowed to fall to the bottom of the dish. The fragment collapsed and the nipple disappeared. When this fragment of tentacle had partially expanded the distal end became more rounded, like the distal cut end of a tentacle whose tip had been cut off after the tentacle was severed from the animal. As in such a distal cut end, the walls were opaque and slightly constricted.

Polarity is shown in the difference of activity of the proximal and distal cut ends. While on the attached stump a temporary muscular nipple was formed within half an hour after cutting, there was no evidence of such closure at the proximal end of the free tentacle (fig. 4, *a*). The end was open, a condition sharply contrasted with that of the closed nipple which marked the other cut end resulting from the same cut. The slight inrolling seen in the stump immediately after cutting was seen here also, but the opening was irregularly outlined and the wall was wrinkled, sometimes deeply so. Figure 4, *a*, shows such an end, six hours after the cut was made. Twenty-three hours after the cut was

made, and when the end of the tentacle stump had become rounded and closed (as in fig. 2), the excised tentacle was still open at its proximal end, but the infolding had increased and the opening had diminished. There had been no contraction of circular muscles, and the wall was strongly wrinkled in a longitudi-

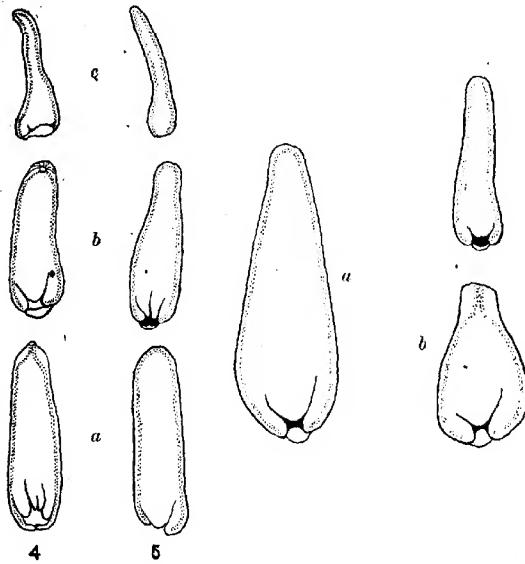


Fig. 4 Excised tentacle cut into three fragments, six hours after cuts were made. The three pieces are placed in their original orientation. $\times 5$.

Fig. 5 Same fragments as in figure 4, twenty-three hours after the cuts were made. $\times 5$.

Fig. 6 Excised tentacle; a, three days after cutting; b, c, the portions of the same tentacle thirty minutes after being cut in two. $\times 5$.

nal direction (fig. 5, a). The wrinkles remained even after the tentacle end became temporarily or permanently closed,—in fact as long as the tentacles were kept alive. The proximal end of the middle piece (b) was also open during the twenty-three hours of observation (figs. 4, 5) and its wall was wrinkled. The opening

was large and its outline angular. The tissue around it was not opaque. The distal end of the inner piece (*a*)—on the opposite side of the same plane of cutting—was smooth. It had, at first, a small round opening and the surrounding tissue was opaque (fig. 4). Twenty-three hours after cutting, it was closed (fig. 5). The proximal end of *c* and the distal end of *b* showed the same contrast, except that the walls of the proximal end of *c* were not so prominently wrinkled. Only in the case of cuts near the tip of the tentacle, where the diameter was very small, did the two openings resulting from the cut become nearer alike in size and the cut ends similar in smoothness; but in no proximal ends did opaque tissue appear, and nothing was found that indicated a reversal of polarity as found in *Condylactis* (Rand, '09, p. 225), where a nipple formed for a short time at the proximal end of the most distal fragment. Figure 6, representing tentacle fragments of a larger animal, illustrates the same polarity. In this case the fragment (*a*) was severed from a tentacle and three days later was divided into the two pieces, *b* and *c*. Thirty minutes after the latter cut was made, the constriction on the distal end of *b* and the open wrinkled appearance at the proximal end of *c* were evident. The two adjacent ends of the fragments, proximal and distal, very evidently react in unlike ways. For, even in those cases where the distal end does not completely close by muscle action, its rounded and smooth surface, its small circular hole and opaque tissue are in contrast with the wrinkled walls, the large irregular opening, and the lack of opaque tissue of the proximal cut end.

A series of twenty excised tentacles, kept in chloretone solutions, was watched. The animal was kept in chloretone until there was no response to touch. A tentacle was then severed and a piece cut away from its distal end. After care had been taken to see that the walls were not held together by the pinching of the scissors, the closure of both proximal and distal ends was watched. As was the case in the attached tentacle stumps in chloretone, all trace of anything like nipple formation was lacking at a distal end. The abrupt initial inrolling was followed by the slow in-bending of the edges toward the center. The opening was reduced

more than one half during eight hours of watching. There were shallow longitudinal wrinkles in the wall, but the distal end had a more rounded outline than the proximal one. After the return to normal sea water (seven to eight hours after the cutting), the distal end became smooth and rounded, as in the normal closure of the distal end of an excised fragment. Some fragments were taken from the chlore-tone solution and fixed. Conditions found in the sections of these fragments are not different, as regards distal ends, from those described for the attached stump in chlore-tone. The proximal end of the pieces in chlore-tone is strongly wrinkled longitudinally and the walls are very deeply inrolled. Except for the extent of the inrolling, no difference exists between the closure of a proximal end in normal sea water and in chlore-tone.

In another series of experiments thirty-six tentacle fragments were sectioned at certain intervals after excision, to corroborate the seeming closure of the distal ends and to see if the proximal ends remained open. In these fragments, as observed alive in a watch glass, some proximal ends were open, but many seemed closed. Eleven fragments lived in watch glasses for two days after excision, when they were fixed; the remaining twenty-five were fixed after four days.

Sections show that the distal ends of all but one were structurally closed. This one had both ends open before fixation and cellular debris streamed from either end. It was evidently in a state of disintegration. Twenty of the thirty-six proximal ends were open, ten were obviously closed, and six doubtfully so. Four of the tentacles, taken from a large animal, were larger than the others, measuring 20 mm. in length and 2.5 mm. in diameter at the base. Size, however, does not seem to be a factor involved; for of the four larger tentacle fragments, two were structurally closed. The ten closed proximal ends show, in the sections, a much stronger inrolling than any distal end (fig. 7). The walls of the proximal ends are brought close together before changes in the thickness and relative position of the layers occur. At the distal end, the cell layers of the very small region of union (*co'jet*, fig. 8) are thin and the cells have no walls or are irregular in shape.

The region of union at the proximal end is similar in extent and character. Around this region of union is one in which the cells of the ectoderm are oblique to the surface of the layer. Nettle cells (fig. 8, *nm'cys.*) and gland cells (*cl. muc.*) occur here, but the nerve layer and mesoglea are not found. This region is very narrow at the proximal end.

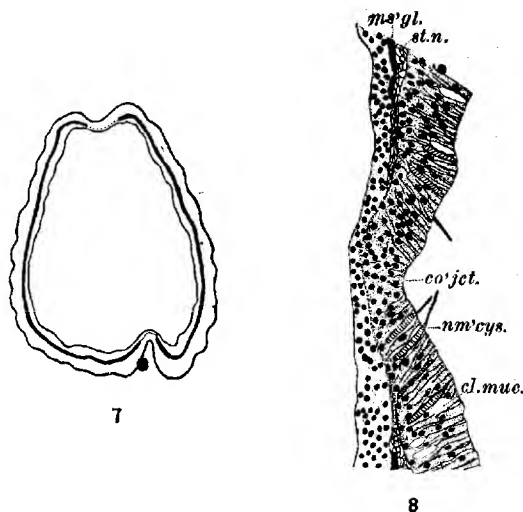


Fig. 7 Camera outline of longitudinal section of excised tentacle whose tip was removed at time of excision. Four days after excision. $\times 30$.

Fig. 8 Distal end of tentacle exhibited in figure 7, showing the region of closure of the tentacle. $\times 190$. *ms'gl.*, mesoglea; *st. n.*, nerve layer; *co'jct.*, region of ectodermic union; *nm'cys.*, nematocyst; *cl. muc.*, mucus cell.

Some grafting experiments were made to see if the polarity is reversible. The Metridium was kept in chloretone until it no longer responded to touch. A tentacle was then cut off near the base and its tip removed. Through the lumen of the tentacle fragment was thrust a bristle, by which it was carried to an attached tentacle which had been cut at such a level that its cut edge was of nearly the same circumference as the edge to be grafted upon it. The apposition of the edges was difficult, because the

tentacle fragment always collapsed and the edges of the tentacle stump rolled in slightly. If the edges were successfully brought together, the bristle was then left in the lumen of the tentacle stump and the fragment, and the fragment was held in place for a time by forceps. To graft to a stump such a tentacle fragment in its normal orientation was less difficult than in the reversed orientation, because the fragment, as when free, tended to move in the direction of its original basal end. As this tendency in the reverse graft was away from the stump, the fragment had to be held to the stump against the action of its cilia.

The graft of a fragment on to an attached stump in normal orientation was made twice successfully. On the following day, the grafted tentacle fragment responded to touch like an excised tentacle, while the stump to which it was grafted was not included in this response unless the stimulus was severe. Twenty-four hours after grafting in one case, forty-eight in the other, the tip of the grafted piece was cut off. Thereupon the cut end closed with the formation of a nipple, as in a normally attached tentacle.

The reverse graft was successfully made only once, and this was not very satisfactory. Whether the proximal end of the fragment, which was reversed in the process of grafting so that it became the distal end of the tentacle, remained open when the animal was returned to normal sea water, could not be determined, since the animal remained contracted for some time after the operation. When the grafted tentacle could be seen, the end was found to be open; but after three days the graft was constricted off and was dead.

Two tentacle fragments cut at both ends were held together on a bristle, or were held in contact between pieces of cover glass. By these means, a number of reverse grafts with the bases together and a few with the tips together were made. Grafts of the former kind were sometimes found among the fragments in the chloretone solution when two fragments, moving as they do in the direction of their bases, had opposed each other long enough for the edges to unite. The grafts were kept in chloretone for eight to ten hours, and thereafter in sea-water. The line of union of the two fragments was marked by a groove. This groove remained

during the time the two fragments were kept under observation, but at the end of the experiments it was proved by study of sections, that the cavities of the grafted pieces were continuous. On each day after union, tests were made to determine the direction of ciliary motion, and the responses to touch. The cilia were not reversed in any of the experiments; but moved in opposite directions in the united fragments. Whether, in response to touch, the tentacle bent toward or away from the point of contact, could not always be determined, but in response to a slight touch, the contraction was always proximal to the point of contact for that graft fragment, while the response did not extend to the other fragment. Finally, when the ends of such grafts were cut off, each grafted part behaved as if it were separate. In the grafts with the basal ends united, the cut tips contracted slightly, and in a few hours became rounded and closed like distal ends of free excised fragments. In the grafts with distal ends united the cut ends continued to behave like proximal ends, remaining open for a long time without constriction, and showing the wrinkles peculiar to proximal ends. In *Metridium*, therefore, no reversal of the ciliary or muscular polarity in the tentacles was found to occur.

SUMMARY

Metridium exhibits the same method of closing a cut tentacle that was found by Rand for other sea anemones. When a part of the tentacle is cut off, the cut edge of the attached stump rolls in slightly as the tentacle collapses. The adjacent tentacles may bend in toward it, and the neighboring region of the disk may be invaginated. As the cut tentacle expands, a small, muscular, nipple-like sphincter closes the end tightly. Within twelve to twenty-four hours after cutting, the tentacle becomes structurally closed and the sphincter is gradually relaxed.

In an excised tentacle whose tip is cut off, the distal cut end becomes rounded and shows, by its shape and change of color, evidence of muscular contraction comparable to that which produces the nipple of the attached stump, although the end does not actually become nipple shaped. If the end of an attached

stump which has formed a nipple is cut off, the nipple loosens and the end which previously bore the nipple assumes the appearance of the distal cut end of an excised tentacle whose tip was cut away after excision of the tentacle.

When the animal is kept in chloretone solution until there is no response to touch, muscle action being therefore eliminated, the cut tentacle does not form a nipple. The initial inbending occurs, and the opening grows gradually smaller by means of a slow radial closure, which is completed in ten to twelve hours. The nipple, therefore, results from muscular contraction.

Polarity is seen to exist in *Metridium* tentacles, not only in the well known fact that the effective stroke of the cilia is always toward the tip, but also in the reaction to touch. This reaction consists in contractions which are proximal to the point of contact. Contraction of a narrow circular zone lying immediately proximal of the point of contact is succeeded by a bend toward, or in some conditions away from, the point of stimulation, and this may be accompanied by a general contraction of the proximal portion of the tentacle. Reactions in the excised tentacle are similar but weaker.

Polarity is shown also by differences in the wound-closing reactions of the proximal and distal ends of excised tentacles. In the distal cut ends the opening is temporarily closed or reduced by a constriction comparable to, but not as pronounced as, that which forms the nipple on an attached stump. The proximal ends show no evidence of such a constriction. A rounded form without wrinkles is characteristic of the distal end. The opening of the proximal end is irregularly outlined and the walls are deeply wrinkled longitudinally. Distal ends become structurally closed and present a smooth, rounded surface. Proximal ends sometimes remain open, but in other cases close. When they close, deep longitudinal wrinkling is characteristic.

Tentacle fragments which were kept in chloretone in order to eliminate the muscle activity close their distal ends by a slow radial movement of the layers of the tentacle wall. The walls of the proximal ends of such fragments are wrinkled longitudinally and are strongly inrolled; they may or may not become closed.

Inverse grafts of two tentacle fragments, base to base, or tip to tip, show no reversion of ciliary action. The reactions to touch, and the closure of cut ends in such grafts are the same as in similar fragments which have not been grafted.

In regard to the character of the mechanism involved in the polarity of muscle action—the proximal muscular response to touch and the temporary closure of a distal wound by a muscular sphincter—my experiments add little to what is already known for other sea anemones. The results do not show with any definiteness to what extent the muscles involved in the movement or closure of the tentacle are dependent on nerve mechanism. This point is fully discussed by Rand with reference to the results of the study of other sea anemones. My results upon *Metridium* are similar to his. We might infer that the *Metridium* tentacle contains very short nerve fibers in which the impulse runs only toward the base of the tentacle, and that there are separate sets of nerve cells for the endodermal circular and the ectodermal longitudinal muscles. But it must be remembered as an argument against the existence of such a simple mechanism that Rand found evidence of a reversal of polarity in distal pieces of *Condylactis* tentacles, where proximal cut ends exhibited temporarily a more or less distinct nipple. If the muscle fibers, and particularly the circular ones, act without nerve control, it is still not clear why there should be one result on one side and another on the opposite side of the same plane of cutting.

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ARTIFICIAL PARTHENOGENESIS AND HYBRIDIZATION IN THE EGGS OF CERTAIN INVERTEBRATES

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INTRODUCTION

The experiments described in the following pages are to be placed in three divisions:

1. Experiments in the production of polar bodies and cleavage in *Cerebratulus* by artificial means.

2. Experiments relating to artificial hybridization between *Cerebratulus* and certain other invertebrates, wherein the combination of the power of the spermatozoön of the foreign animal and of the efficient parthenogenetic reagents discovered in the first set (1) of experiments was utilized, to induce hybridization, where the influence of the sperm alone was found to be inefficient.

3. Experiments which may be described as by-products of the principal work, such as the following: (a) Effect of sperm extract; (b) Effect of the phospholipine, 'lecithin' in inducing changes in unfertilized eggs of *Arbacia*; (c) Rôle of hydrogen and hydroxyl ions in artificial parthenogenesis.

The conclusions which seem to be valid from these experiments are as follows:

1. *Cerebratulus lacteus* and *marginatus* resemble several other invertebrates in their refractoriness in response to reagents which readily induce artificial parthenogenesis in certain forms of animals.

2. When reagents are at all effective, they produce polar body formation¹ and early morulas, but none were found to cause farther development.

3. Such reagents as are effectual in causing even this development in the unfertilized egg aid artificial hybridization with foreign spermatozoa, as far as could be determined, in no manner.

4. Artificial hybridization was found to be possible in but one instance, namely, the spermatozoa of the mollusk, *Ilyanassa obsoleta*.

5. Extract of sperm, made by killing the spermatozoa at 40°C., did not induce egg development in *Cerebratulus*, nor in *Arbacia*.

6. Lecithin from the hen's egg and from eggs and spermatozoa of *Arbacia* produced no effect upon the eggs of *Arbacia*.

7. The conclusions of Jacques Loeb concerning the rôle of *H* and *OH* ions in artificial parthenogenesis are favored by these experiments.

MATERIAL AND METHODS

The work was primarily planned to cover section 2 of the above sets of experiments, using the clam worm found so readily in full breeding season at the Harpswell Laboratory, South Harpswell, Maine, during the summer months, from June until September, but as explained above, it was necessary first of all to study in detail the various reagents which have been used effectually in other instances, but not at all, as far as I know, upon any nemertean. In order to corroborate the work upon material from an entirely different locality and under entirely different circumstances as to environment, etc., a study was made of the clam-worm found at New Haven, Connecticut, which is sexually mature during the months of March or April, according to the season. Finally, the work upon *Arbacia* and some other forms was carried on at the Marine Biological Laboratory, Woods Hole, Massachusetts.

I am greatly indebted to the officers directing the three laboratories at which I worked, namely, the Harpswell Laboratory,

¹ The nucleus of the egg prior to fertilization rests in the metaphase of the first polar body formation until the spermatozoön enters, when the mitosis proceeds through anaphase and telophase.

the Marine Biological Laboratory and the Sheffield Laboratories at New Haven. My thanks are especially due to Prof. Wesley R. Coe, of Yale University, whose wide acquaintance of the breeding habits and cytology of *Cerebratulus* materially aided in my work. I am indebted, likewise, to Prof. Jacques Loeb and his associates at the Rockefeller Institute Laboratory at Woods Hole, who placed at my disposal an abundant supply of sea-urchins' ovaries and testes, which were not demanded in the work of Professor Loeb's Laboratory. Prof. Ralph Lillie and Dr. J. F. McClendon likewise contributed a supply of similar material, without which I could not have obtained sufficient lecithin for the work with this compound.

The methods used in the different experiments naturally vary according to the procedure, but it may be of advantage to describe the principal ones which are applicable throughout.

Fresh-water (sweet-water) was used throughout to sterilize instruments and dishes from contaminating spermatozoa and recourse to heat or chemical sterilization was avoided as hindering the experiments in time and in danger of introducing a variable wholly apart from any which might come in from natural sources. The Harpswell Laboratory is so situated that the purest of sea-water free from spermatozoa of *Cerebratulus* may be readily obtained by taking a motor boat off shore for a mile or so at flowing tide. Moreover, in this laboratory, the temperature was low (on the table upon which the finger-bowls were placed, the air current seldom ran above 17°C.) and it was only exceptionally necessary to resort to ice-boxes. Hence, the normal environmental factors were kept as nearly as possible throughout these experiments. There are no large cities near the Harpswell Laboratory and the conditions as far as contamination with decaying organic matter which might modify results, are not to be considered.

The worms were taken at about three-day intervals and the females and males were kept isolated in battery jars beneath the laboratory, where the temperature was even lower than in the work rooms above. It was found necessary to isolate not only males from females, but the individuals in every case from other individuals, owing to the fact that the sex products were shed

when the individuals were allowed to mingle and through their disintegration the water fouled and the specimens suffered.

Conditions at New Haven were quite different, which is a valuable check to the Maine experiments. Here the worms live in sea-water which has a large fresh-water dilution. Moreover, the sewage from the city must exert a decided influence in altering the character of the sea-water.

Finally, at Woods Hole, there was an unfortunate dearth of favorable material of *Arbacia*, during the season of 1911, which did not permit of as extended a series of observations as otherwise. Only eggs where the check gave decidedly favorable results were used and at times it was found difficult to procure many individuals answering this requirement.

Environmental conditions

Certain determinations of environmental conditions were made which may be of some value in regard to the experiments conducted upon the same material (*Cerebratulus*) but at different times and places. I shall present these data in table on page 475.

It will be seen that the water at Woods Hole and at New Haven run similarly, while the Harpswell water has a higher acid content (or lower *OH* content). Whether this difference is due to the sewage in these cases, I cannot determine, but I should be inclined to assume that this is not the case, at least at Woods Hole, for the water from Vineyard Sound is comparatively clean, judging from determinations of nitrates and nitrites and other factors determined by the United States Bureau of Fisheries survey of 1904-09. It is probably a matter of normal constituents. The water at Harpswell comes partly from the cold Arctic Current² which bathes the Gulf of Maine, while the Long Island and Vineyard Sound water comes largely from the Gulf Stream.

Such are some of the environmental conditions which may have some bearing upon the following experiments with *Cerebratulus*.

²Oceanographers are abandoning the idea of a current of this nature and explain the cold water of the Gulf of Maine as coming from cold depths.

The data for Woods Hole were put in for comparison, but with no direct bearing upon the present problems.

ENVIRONMENTAL CONDITION	NEW HAVEN	HARPSWELL	WOODS HOLE
Sea-water density.....	1.0195 ¹	1.0238 ²	1.024
Temperature.....	14°C. ³	15°C.	18°C.
Alkalinity of sea-water ⁴ as determined by reaction to:			
Phenolphthalein...	{ distinctly red ⁵ = $N10^{-12}$	colorless = $N10^{-11}$	distinctly red ⁶ = $N10^{-13}$
Neutralrot.....	{ yellow = $N10^{-12}$	orange = $N10^{-11}$	red ⁷ = $N10^{-10}$

¹ On the Gay-Lussac hydrometer scale, double glass-distilled water at 15°C. giving 1000. The New Haven density was taken from water drawn from the sea at Sagin Rock, at the site where the Yale University Laboratory for marine work is to be erected.

² Read off Haskell's Island on flood-tide by Prof. Loring Barrows, Department Geology, Trinity College, to whom the writer is indebted for the kindness.

³ Taken at New Haven, April 27, 1912, at Harpswell, surface of open sea off Haskell's Island, 16.5 at 9.00 and Woods Hole July 6, Government Wharf at the U. S. B. F., at 12.00. Time data will be expressed in this paper from 1.00 to 24.00, beginning at midnight.

⁴ Determined by titration with indicators. This, of course, gives only the metal-replaceable hydrogen and not the true hydrogen ion content (see Huber '09 and Friedenthal '10), which can be determined by conductivity determination. There is doubtless considerable difference between these two factors in sea-water where sewage brings in organic matter and where the CO₂ component must vary widely. Unfortunately, no conductivity determinations were possible at Harpswell and none have been made elsewhere. For this reason, no comparison could be made unless such were the case. It may be explained that by 'alkalinity' is meant the preponderance of alkali metals (Na, Mg, K, Ca) which are united to CO₂ and is quantitative expressed by the older method of Törnøe '76 in cubic centimeters of CO₂; but compare Fox '09.

⁵ Friedenthal '10, p. 546.

⁶ Quoted from Loeb '09, p. 45, the determination made 'im Laboratorium,' but Dr. J. F. McClendon writes me that water taken from the U. S. B. F. wharf gives a faint rose color with phenolphthalein, thus making the OH content $N10^{-12}$ or even lower.

⁷ McClendon, by letter.

TABLE 1
Table of summaries: Data of experiments upon artificial parthenogenesis

SPECIES	LOCALITY	DATE	PREVIOUS TREATMENT	AFTER TREATMENT	RESPONSE
Cerebratulus	Harpowell	7-30-10	$\frac{N}{10}$ HCl 15 cm. ³ + 85 sea-water, 5 minutes	To sea-water	Polar bodies
Cerebratulus	Harpowell	7-30-10	$\frac{10}{10}$ HCl with KCl in small amount	To sea-water	Polar bodies
Cerebratulus	Harpowell	8-1-10	$\frac{N}{10}$ HCl 15 cm. ³ + 85 sea-water, 5 minutes	To sea-water	Polar bodies
Cerebratulus	Harpowell	8-1-10	$\frac{N}{10}$ HCl 15 cm. ³ + 85 sea-water, 6 minutes	To sea-water	Polar bodies
Cerebratulus	Harpowell	8-2-10	$\frac{N}{10}$ HCl 10 cm. ³ + 90 sea-water, 9 minutes	To sea-water	None
Cerebratulus	Harpowell	8-2-10	$\frac{10}{10}$ HCl 12 cm. ³ + 88 sea-water, 5 minutes Again at interval of 3 hours	To sea-water, fresh	
			One portion of eggs then placed in sea-water oxygenated from oxygen tank, which gave		Irregular segmentation after polar bodies
Cerebratulus	Harpowell	8-2-10	$\frac{N}{10}$ HCl 17 cm. ³ + 83 sea-water, 5 minutes Polar bodies irregular. Changed sea-water at interval of 3 hours and placed portion of eggs in oxygenated sea-water, which gave	To sea-water	Polar bodies
Cerebratulus	Harpowell	8-2-10	$\frac{N}{10}$ HCl 10 cm. ³ + 90 sea-water, 5 minutes To sea-water deoxygenated (by boiling and cooling hermetically sealed), giving After interval of 4 hours, oxygenated from tank, giving	Delayed	Irregular segmentation
Cerebratulus	Harpowell	8-2-10	$\frac{N}{10}$ HCl 15 cm. ³ + 88 sea-water, 5 minutes Then after interval of 24 hours divided, one part to oxygenated sea-water, giving to de-oxygenated sea-water, giving	To sea-water	Polar bodies 2-cell stage Polar bodies Irregular segmentation None

Cerebratulus	Harpwell	8-2-10	$\frac{N}{16}$ HCl 10 cm. ³ + 90 sea-water, 5 minutes Bubbled O-gas from cylinder again at second polar body formation for 10 minutes. Then Again bubbled O-gas after interval of 4 hours $\frac{N}{16}$ HCl 15 cm. ³ to 85 sea-water, 5 minutes To oxygenated sea-water with changes and placed at 10°C.	To O-sea-water	Polar bodies
Cerebratulus	Harpwell	8-3-10			None 2-cell stage
Cerebratulus	Harpwell	8-4-10	$\frac{N}{16}$ HCl 15 cm. ³ + 85 sea-water, 8 minutes (17°C.)	To sea-water	None Irregular segmen- tation
Cerebratulus	Harpwell	8-4-10	$\frac{N}{16}$ HCl 15 cm. ³ + 85 sea-water, 5 minutes (17°C.) $\frac{N}{16}$ HCl 15 cm. ³ + 85 sea-water, 8 minutes (19.5°C.)	To sea-water To sea-water	Morulas Irregular divi- sion
Cerebratulus	Harpwell	8-4-10	$\frac{N}{16}$ HCl 15 cm. ³ + 50 sea-water, 5 minutes (28°C.)	To sea-water	Irregular segmen- tation
Cerebratulus	Harpwell	8-5-10	$\frac{N}{16}$ HCl 15 cm. ³ + 85 sea-water, 5 minutes After interval of 2½ hours, into CO ₂ -water from cylinder for 2½ hours. Then	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-5-10	$\frac{N}{16}$ HCl, 15 cm. ³ + 85 sea-water, 5 minutes. KCl (17cm. ³ + 83 sea-water)	To O-less sea-water	Morulas.
Cerebratulus	Harpwell	8-8-10	$\frac{N}{58}$ KCl 10 + 50 sea-water + $\frac{N}{16}$ NaOH, 2 cm. ³ for 68 minutes. Then	To sea-water after 45 minutes	Polar bodies
Cerebratulus	Harpwell	8-8-10	$\frac{N}{58}$ KCl 10 cm. ³ + 50 sea-water + $\frac{N}{100}$ NaOH, 0.5 cm. ³ for 57 minutes	To sea-water	None
Cerebratulus	Harpwell	8-8-10	$\frac{N}{58}$ KCl, 10 cm. ³ + 50 cm. ³ sea-water + $\frac{N}{16}$ NaOH, 1 cm. ³ for 66 minutes	To sea-water	None
Cerebratulus	Harpwell	8-8-10	$\frac{N}{58}$ KCl, 10 cm. ³ + 50 cm. ³ sea-water + $\frac{N}{100}$ NaOH, 5 cm. ³ for 60 minutes	To sea-water	None
Cerebratulus	Harpwell	8-8-10	$\frac{N}{58}$ KCl, 10 cm. ³ + 50 cm. ³ sea-water + $\frac{N}{100}$ NaOH, 0.2 cm. ³ for 56 minutes.	To sea-water	None

TABLE 1—Continued

SPECIES	LOCALITY	DATE	PREVIOUS TREATMENT	AFTER TREATMENT	RESPONSE
Cerebratulus	Harpowell	8-8-10	$\frac{58}{8}$ KCl, 10 cm. ³ + 50 cm. ³ sea-water + $\frac{N}{10}$ NaOH, 0.1 cm. ³ for 52 minutes (20.5°C.)	To sea-water	None
Cerebratulus	Harpowell	8-13-10	$\frac{58}{8}$ KCl, 15 cm. ³ + 85 sea-water	To sea-water	None
Cerebratulus	Harpowell	8-13-10	$\frac{58}{8}$ KCl, 10 cm. ³ + 90 cm. ³ sea-water	To sea-water	None
Cerebratulus	Harpowell	8-13-10	$\frac{58}{8}$ KCl, 5 cm. ³ + 95 sea-water	To sea-water	None
Cerebratulus	Harpowell	8-13-10	$\frac{58}{8}$ KCl, 25 cm. ³ + 75 sea-water	To sea-water	None
Cerebratulus	Harpowell	8-10-10	$\frac{58}{8}$ MgCl ₂ , 25 cm. ³ + 2.5 cm. ³ sea-water, 1 hour	To sea-water	None
Cerebratulus	Harpowell	8-10-10	Above diluted one-half	To sea-water	None
Cerebratulus	Harpowell	7-29-10	$\frac{N}{10}$ NaOH, 1.5 cm. ³ + 50 cm. ³ sea-water, 1 hour	To sea-water	None
Cerebratulus	Harpowell	7-30-10	$\frac{N}{10}$ NaOH, 3 cm. ³ + 50 cm. ³ sea-water, 1 hour	To sea-water	None
Cerebratulus	Harpowell	8-1-10	$\frac{N}{10}$ NaOH, 4 cm. ³ + 50 cm. ³ sea-water, 1 hour	To sea-water	None
Cerebratulus	Harpowell	8-1-10	$\frac{N}{10}$ NaOH, 1.0 cm. ³ + 50 cm. ³ sea-water, 1 hour	To sea-water	None
Cerebratulus	Harpowell	8-1-10	$\frac{N}{10}$ NaOH, 2.0 cm. ³ + 50 cm. ³ sea-water, 1 hour	To sea-water	None
Cerebratulus	Harpowell	8-1-10	$\frac{N}{10}$ NaOH, 0.5 cm. ³ + 50 cm. ³ sea-water, 1 hour	To sea-water	None
Cerebratulus	Harpowell	8-1-10	$\frac{N}{10}$ NaOH, 1 cm. ³ + 50 sea-water oxygenated, by bubbling, 33 bubbles per minute for 40 minutes	To sea-water	None
Cerebratulus	Harpowell	8-2-10	In pure sea-water with current of oxygen gas from cylinder for 10 minutes. Repeated after interval of 60 minutes for 30 minutes		Irregular 2-cell and 4-cell stage
Cerebratulus	Harpowell	8-3-10	In boiled sea-water cooled in hermetically sealed vessel (19°C.) under constant current of CO ₂ -gas from cylinder for 28 minutes		None
Cerebratulus	Harpowell	8-1-10	Ten minutes exposure to current oxygen		None
Cerebratulus	Harpowell	7-26-10	In CO ₂ -water for 60 minutes	To pure sea-water	2-cell and 4-cell stage
Cerebratulus	Harpowell	7-26-10	In CO ₂ -water for 60 minutes	To pure sea-water	None

Cerebratulus	Harpwell	7-28-10	10 cm. ³ sea-water through which CO ₂ is bubbled from Kipp generator (wash) 5 minutes to pure sea-water and after 60 minutes added 10 cm. ³ KCl $\frac{3}{2}$ to 50 cm. ³ sea-water. Then	To pure sea-water	None
Cerebratulus	Harpwell	7-28-10	As above, but 12 cm. ³ KCl solution used	To sea-water	None
Cerebratulus	Harpwell	8-3-10	Into CO ₂ -water at 5°C.	To O-less sea-water	None
Cerebratulus	Harpwell	8-3-10	CO ₂ -water, 14°-9° C.		None
Cerebratulus	Harpwell	8-4-10	CO ₂ -water (1 hour bubbling of CO ₂ -gas through 50 cm. ³ sea-water) for 10 minutes. Then into KCl $\frac{3}{2}$, 10 cm. ³	To sea-water	Irregular segmentation
Cerebratulus	Harpwell	8-4-10	As above, but exposed to CO ₂ -water, 5 minutes		None
Cerebratulus	Harpwell	8-4-10	As above, but exposed to CO ₂ -water, 15 minutes		None
Cerebratulus	Harpwell	8-2-10	As above, but exposed to CO ₂ -water, 20 minutes		None
			Eggs subjected to the following rise in temperature in thermostat:		
			Time		
			14:54.....	°C.	
			14:59.....	22	
			15:02.....	2	
			15:25.....	27	
			15:55.....	30	
			16:20.....	28	
			17:00.....	32	
			17:13.....	28	
			17:13.....	32	
Cerebratulus	Harpwell	8-3-10	This gave		None
			In sea-water placed in freezing mixture (10°C. 0°C.) for 60 minutes		None
Cerebratulus	Harpwell	8-3-10	In oven at 20°C. for 120 minutes		None

TABLE 1—Continued

SPECIES	LOCALITY	DATE	PREVIOUS TREATMENT	AFTER TREATMENT	RESPONSE
Cerebratulus	Harpwell	8-4-10	oven at 21° and then gradual rise through 4 hours to 40°C.		None
Cerebratulus	Harpwell	8-12-10	Left 12 hours at 12°C. Then into sea-water at 18°C.		None
Cerebratulus	Harpwell	8-20-10	CH ₃ COOH, $\frac{1}{10}$ 15 cm. ³ + 85 sea-water, 5 minutes		None
Cerebratulus	Harpwell	8-6-10	$\frac{1}{10}$ butyric, 2.8 cm. ³ to 50 cm. ³ sea-water, 2 minutes	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 10 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 9 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 8 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 7 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 6 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 5 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 4 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 3 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with 2 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-6-10	As before, with .1 cm. ³ butyric	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-11-10	$\frac{1}{10}$ (COOH) ₂ , 6 cm. ³	To sea-water	None
Cerebratulus	Harpwell	8-11-10	As before, 8 cm. ³ oxalic, 5 minutes	Slight cytology-	None
Cerebratulus	Harpwell	8-11-10	As before, 2 cm. ³ oxalic	To sea-water	None
Cerebratulus	Harpwell	8-11-10	As before, 4 cm. ³ oxalic	To sea-water	None
Cerebratulus	Harpwell	8-11-10	As before, 10 cm. ³ oxalic	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-11-10	As before, 12 cm. ³ oxalic	To sea-water	Few polar bodies
Cerebratulus	Harpwell	8-13-10	$\frac{1}{10}$ tartaric (d) 0.5 cm. ³ to 250 cm. ³	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-13-10	As before, 1 cm. ³ 1 minute in O-sea-water	To sea-water	None
Cerebratulus	Harpwell	8-13-10	As before, 1 cm. ³ 1 minute	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-13-10	As before, 1 cm. ³ 2 minutes	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-13-10	to $\frac{1}{10}$ CaCl ₂ 20 cm. ³ to 80 bodies	To sea-water	Polar bodies

Cerebratulus	Harpowell	8-13-10	As before, 1 cm. ³ 2 minutes. Then to $\frac{N}{10}$ NaOH after polar bodies 2 cm. ³ NaOH 50 cm. ³	To sea-water	Polar bodies None
Cerebratulus	Harpowell	8-13-10	As before, 2 cm. ³ Too strong.		
Cerebratulus	Harpowell	8-13-10	As before, 0.75 cm. ³ to 250 cm. ³ sea-water 2 minutes. Then into CO ₂ -water at 19°C., giving		Polar bodies
Cerebratulus	Harpowell	8-13-10	As before, 1 cm. ³ for 3 minutes. After polar bodies into $\frac{N}{10}$ KCl 25 cm. ³ to 75 cm. ³ sea-water. Two lots:		
			At 19°C., giving		
Cerebratulus	Harpowell	8-13-10	At 7°C., giving		Polar bodies
			As before, 1 cm. ³ for 2 minutes. After polar bodies to saponin, 0.5%, 4 drops to 5 cm. ³ sea-water, 2 minutes		Polar bodies
Cerebratulus	Harpowell	8-13-10	As before, 0.75 cm. ³ to 250 cm. ³ sea-water		Polar bodies
Cerebratulus	Harpowell	7-29-10	Saponin 8 drops to 5 cm. ³ sea-water, 4 minutes	To sea-water	Polar bodies
Cerebratulus	Harpowell	7-27-10	Saponin as before, 5 minutes. Then to KCl 2.5N (12 cm. ³ to 30 cm. ³ sea-water) for 60 minutes	To sea-water	Polar bodies
Cerebratulus	Harpowell	7-30-10	Saponin as before, 15 drops, 11 minutes. To sea-water. To KCl 15 cm. ³ after 38 minutes		None
Cerebratulus	Harpowell	7-30-10	Saponin as before, 8 drops. To sea-water. To KCl		None
Cerebratulus	Harpowell	8-1-10	Saponin as before, 8 drops, 10 minutes	To sea-water	None
Cerebratulus	Harpowell	8-3-10	Saponin as before, 8 drops	To boiled sea-water	None
Cerebratulus	Harpowell	8-1-0	Saponin as before, 7 drops, 5 minutes	To sea-water	None
Cerebratulus	Harpowell	8-4-10	Saponin as before, 9 drops, 6 minutes	To sea-water	None
Cerebratulus	Harpowell	8-4-10	Saponin as before, 5 drops, 5 minutes	To sea-water	Polar bodies and irregular divisions.
Cerebratulus	Harpowell	8-4-10	Saponin as before, 11 drops, 20 minutes	To sea-water	None

¹ Saponin used throughout in 0.25% (25.0 cg. Merck saponin powder up to 100 cm.³ sea-water).

² The experiments at New Haven were continued with saponin, to determine how differently the eggs responded from those at Harpswell. The amount of saponin varied from 2 drops to 20 drops, with results exactly similar to those given for the Harpswell specimens; therefore, I shall not enumerate these details. No experiments conducted gave segmentation stages, although polar bodies formed readily.

Discussion of experiments dealing with artificial parthenogenesis

Table 1, showing the details of the experiments which were conducted to show what the behavior of *Cerebratulus* eggs would be to the reagents which have been used in artificial parthenogenesis, may be summarized as follows:

1. The reagent most successful and most certain of results was found to be saponin, which has been introduced into this work by Jacques Loeb ('98). The power of this amorphous glucoside is doubtless wholly that of a dehydrating agent, absorbing water from the eggs and therefore not chemical in its action.

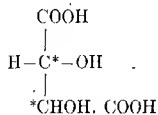
2. The combination of saponin to induce maturation and some of the other agents to carry segmentation farther than the 2 or 4-cell stage gave no favorable results, although a large number were tried, including CO_2 (which is efficient in producing the early morula stages, but none farther); hypertonic solutions, with NaCl , KCl , MgCl_2 , CaCl_2 ; acids, mineral and organic, of the latter, both mono-basic and bi-basic; oxygen and the lack of oxygen, etc.

3. Among other reagents which induced artificial parthenogenesis are HCl , butyric acid, oxalic acid, tartaric acid. Of these organic acids, Loeb's theory concerning the rôle of the OH ion seems to be carried out as far as they are concerned, for the higher ones go from the mono-basic butyric, through dibasic oxalic acid to the derivative di-hydroxy-acid³ the *d*-tartaric, the more efficient

³ The relations between these acids may be presented here for reference:

(Acetic acid) $\text{CH}_3\text{.COOH}$
 Butyric acid $\text{CH}_3\text{.CH}_2\text{.CH}_2\text{.COOH}$
 Oxalic acid COOH.COOH
 (Succinic acid) $\text{COOH.CH}_2\text{.CH}_2\text{.COOH}$ (to show relations of)
 Tartaric acid $\text{COOH.CH}_2\text{O.CH}_2\text{O.COOH}$

Tartaric acid, according to the principle of LeBel-van't Hoff must exist in four different forms, since it has two asymmetrical carbon atoms (asymmetrical atoms marked *)



The commercial tartaric is dextro-rotary. Whether its effectiveness in artificial parthenogenesis has anything to do with its optical activity, is not known, but it is possible.

the acids become, if carbonic acid, H_2CO_3 , be excepted, which is wholly hypothetical as a member of the group to which the others belong.

4. Temperature, mechanical agitation (which was not mentioned) and environmental conditions other than what have been enumerated were not found to be efficient in causing even the expulsion of polar bodies. It was not found possible to note any constant variable between the Harpswell conditions and those at New Haven, although the differences were as great as one could desire for the experiments.

5. There is reason to believe that a strong cytolyzing reagent, like HCl or saponin, followed by a weaker one, such as CO_2 is efficient in carrying out segmentation. However, it was not possible to carry the segmentation by any means beyond the early morula stages. There is clearly here a difference between maturing and segmentation-inducing reagents.

It will be seen that the experiments to be described upon artificial parthenogenesis plus the action of foreign spermatozoa are not easily confused as to the factor at work, for in all cases where the artificial parthenogenetic reagents were used alone (as in the above experiments) the development progressed only to the early morula stages. Hence, if the spermatozoa of the foreign individual exerted any effect, it would be readily recognized, if the effect were at all worthy of notice, that is, efficient in producing an approach to normal development wherein the segmentation proceeded past the early morula stages.

As stated in a previous footnote, the polar bodies of *Cerebratulus* are not expelled until the spermatozoön enters and in this respect, it resembles other animals which have been examined with respect to artificial parthenogenesis, such as the annelid, *Polynoë* (*Lepidonotus*) (Loeb '08); *Chaetopterus* (Mead '98); *Thalassema* (Lefevre '07) and others. Compared to them, however, in regard to the reagents which bring about the whole or completion of maturation, there is a great difference as far as my observations are concerned. In regard to *Polynoë*, Loeb found that "die Eier von *Polynoë* können aber auch im Seewasser ohne Spermatozoön zur Reifung gebracht werden, wenn man dem See-

wasser etwas NaHO zusetzt, nämlich etwa 1.5 ccm. $\frac{N}{4}$ NaHO zu 50 ccm. Seewasser" (Loeb '09 a, p. 158), which is distinctly not possible with *Cerebratulus* in my experience. Nor in regard to *Chaetopterus*, which Mead (l.c.) used at the very beginning of the work upon artificial parthenogenesis, can it be said that it resembles *Cerebratulus* in so far as reagents are concerned which are efficient to induce development, for the addition of a small amount of KCl is sufficient to start the spindle forming the configurations of the anaphase and telophase of the first maturation division.⁴ In the third case, *Thalassema*, it will be recalled that Lefevre (l.c.) found that the following reagents induced development, both maturation and segmentation: HNO_3 , HCl , H_2SO_4 , $(\text{COOH})_2$, CH_3COOH and CO_2 ; although the approach is much nearer to *Cerebratulus*, yet there is a decided difference, since Lefevre readily obtained trochophore larvae from 6 to 60 per cent. Again, these reagents were efficient in themselves, while in *Cerebratulus*, no development was obtained without a *combination of reagents*, except in a very few cases of saponin, used alone; but here, no farther development than the 4 or 8-cell stage was observed.

Whatever the difference is—whether it be temperature, alkalinity, the nature of the egg itself, being accustomed to lower temperatures wherever it occurs than the other examples considered above, so that the membrane may have become highly impermeable to ordinary reagents, and the egg modified to withstand untoward conditions—the fact remains that it is a difficult, and in the light of the experiments described above, impossible task to cause the development to proceed to larval stages.

Experiments upon artificial hybridization

The experiments which I have described in table 2 are somewhat misleading. Only one kind of each of the variations in method of procedure is given and I have not made the complication greater by the addition of any data as to the frequency of error.

⁴ Godlewski ('11) however found that hypertonic solutions produced no effect in this annelid.

Briefly this may be stated as follows: With *Ilyanassa*, the errors were fewest (per cent of error, 80), with *Mytilus* next, but with a far greater number of errors (approximately 95 per cent) while with *Echinarachnius*, although the cell-divisions progressed as far as in *Ilyanassa*, yet the frequency of error was very large (only a few developed in any one batch, but unfortunately the data in percentages were not kept). No success was had with *Phascolosoma*.

In the case of *Arbacia*, which differs in the manner of maturation from *Cerebratulus*, the eggs maturing in the ovary, attempted crosses with *Mytilus* and *Modiolus* were not successful, although the experiments were given a great number of trials. The time of applying the foreign sperm after the eggs were taken from the ovary varied in the several cases, so that here again deference was made to a possible 'refractory period.'

The experiments outlined above are similar in many ways to those which have been done by Steinbrück ('02), Loeb ('07, '09a), Hagedoorn ('09), Godlewski ('05, '11), Kupelwieser ('09), Baltzer ('10), Tennent ('10), and Bataillon ('09), where the eggs of a number of echinoderms, mollusks, etc., were investigated with a view to hybridization, with excellent results in several cases. Some of these investigations pointed to the fact that even when development proceeded after the addition of the foreign sperm, nuclear fusion apparently did not take place, so that the action of the sperm was that of giving an impetus to mitosis in the female pronucleus, the male pronucleus disappearing, with the result that larvae were maternal throughout. This was not true for Baltzer's and Godlewski's experiments. Although cytological material was preserved, the paucity of successes deterred me from making sections of the eggs to determine whether the results were similar to those of the investigators named above and especially Godlewski, Baltzer and Kupelwieser, who made sections of the eggs in various stages.

While there is absolutely no question as to the results which Kupelwieser, Godlewski and others obtained, it seems somewhat strange that two species, *Cerebratulus* and *Arbacia* should respond so slightly to the spermatozoa of other species, some of which

TABLE 2
Table of summaries: Experiments upon artificial hybridization

SPECIES	LOCALITY	DATE	FOREIGN SPECIES	PREVIOUS TREATMENT	AFTER TREATMENT	RESULT
Cerebratulus ♂	Harpwell	7-23-10	Echinarachnius ♀	Small amount sea-water KCl, $3\frac{3}{4}$ 10 cm. ³ A number of these experi- ments were conducted with no favorable result		None
Cerebratulus ♂	Harpwell	7-25-10	Echinarachnius ♀	Small amount sea-water MgCl ₂ $3\frac{3}{4}$ 15 cm. ³ to 50 cm. ³ sea-water	To sea-water	Few irregular divisions
Cerebratulus ♀	Harpwell	7-28-10	Phascolosoma ♂	Sperm diluted in this experi- ment Small amount sea-water. Di- vided into: Lot a. Saponin, short expo- sure, and then to KCl- water Lot b. CO ₂ -water, 13 min- utes exposure Lot c. KCl and then Saponin, 5 drops in 5 cm. ³ sea- water for 9 minutes. Di- vided into two lots for after treatment: 1. Into KCl $3\frac{3}{4}$ 12 cm. ³ + 50 cm. ³ sea-water 2. Into pure sea-water di- rect	To sea-water To sea-water To sea-water	None None None
Cerebratulus ♀	Harpwell	7-28-10	Phascolosoma			None

				Saponin This is a check to the following:	To sea-water	None
Cerebratulus ♀	Harpwell	7-27-10	Ilyanassa	Saponin, 5 minutes	To sea-water	Polar bodies None
Cerebratulus	Harpwell	7-27-10	Ilyanassa ♂	Sea-water small amount KCl	To sea-water	Polar bodies None
Cerebratulus	Harpwell	7-27-10	Ilyanassa	Saponin short exposure	To sea-water	None
Cerebratulus	Harpwell	7-27-10	Ilyanassa	Check gave no development	To sea-water	None
Cerebratulus	Harpwell	7-26-10	Echinarachnius	Small amount sea-water CO ₂	To sea-water	None
Cerebratulus	Harpwell	8-2-10	Ilyanassa	HCl (10 cm. ³)	To sea-water	Irregular segmentation
Cerebratulus	Harpwell	8-1-10	Mytilus	NaOH 0.5 cm. ³ + 50 H ₂ O	To sea-water	None
Cerebratulus	Harpwell	8-1-10	Mytilus	NaOH 0.8 cm. ³ + 50 cm. ³ H ₂ O	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-1-10	Mytilus	NaOH 0.6 cm. ³ + 50 cm. ³ H ₂ O	To sea-water	None
Cerebratulus	Harpwell	8-1-10	Mytilus	Small amount sea-water oxygenated	To sea-water	None
Cerebratulus	Harpwell	8-1-10	Mytilus	Small amount sea-water	To sea-water	None
Cerebratulus	Harpwell	8-2-10	Ilyanassa	NaOH 1.5 cm. ³ to 50 sea-water	To sea-water	None
Cerebratulus	Harpwell	8-2-10	Ilyanassa	CH ₃ COOH 1.5 cm. ³ to 50 sea-water. To sea-water after 6 minutes. Then sperm after 22 minutes	To sea-water	Polar bodies
Cerebratulus	Harpwell	8-2-10	Ilyanassa	Saponin	To sea-water	None
Cerebratulus	Harpwell	8-12-10	Phascosoloma	NaOH 1.5 cm. ³	To sea-water	None
Cerebratulus	Harpwell	8-12-10	Phascosoloma	In small amount own serum	To sea-water	None
Cerebratulus	Harpwell	8-12-10	Phascosoloma	Saponin 2 drops in 5 cm. ³ sea-water	To sea-water	None
Cerebratulus	Harpwell	8-12-10	Phascosoloma	(COOH) ₂ , 4 minutes. Then sperm	To sea-water	None
Cerebratulus	Harpwell	8-12-10	Phascosoloma	(COOH) ₂ , 6 minutes. Then sperm	To sea-water	None

TABLE 2—Continued

SPECIES	LOCALITY	DATE	FOREIGN SPECIES	PREVIOUS TREATMENT	AFTER TREATMENT	RESULT
Cerebratulus	Harpwell	8-12-10	Phascolosoma	(COOH) ₂ , 10 cm. ³ , 8 minutes. Then sperm after 17 minutes.		None
Cerebratulus	Harpwell	8-12-10	Phascolosoma	(COOH) ₂ producing polar bodies. Then after maturation, added sperm		None
Arbacia	Woods Hole	7-20-11	Mytilus	Eggs in 5 cm. ³ sea-water sperm of Mytilus not diluted		None
				Check normal. A number of duplicates were made of this sort		
Arbacia	Woods Hole	7-21-11	Modiolus	As before		None
Arbacia	Woods Hole	7-22-10	Mytilus	A few eggs placed in a watch-crystal and concentrated sperm from Mytilus added (washed and left in pure sea-water)		None

having been used in successful cases (*Mytilus*).⁵ I have repeated Kupelwieser's experiments in a manner as nearly like his own as I could, judging from the printed account without any suggestion of the success, as far as echinoderm-mollusk crossing is concerned, which he experienced, but terminating without the production of later stages than morulas, even when artificial parthenogenetic media were applied.

With the exception of *Ilyanassa*, whose eggs were not ripe at the time the experiments were performed at Harpswell, checks were employed to insure that the spermatozoa of the foreign species were functional and normally so. However, in the case of *Ilyanassa*, the spermatozoa were highly active under the microscope and there is little reason to doubt that they were functional.

There are many other species of invertebrates which show a decided obstinacy to both artificial parthenogenetic reagents and to crossing, although failures are seldom reported, which leads to difficulties for other investigators. • Prof. E. G. Conklin of Princeton University and Dr. J. F. McClendon of Cornell University Medical College have testified to me personally that they have tried several forms which did not respond in any way to artificial reagents. Thus, ascidians seem to be in this category and likewise some of the mollusks, *Mytilus* eggs having been given a good test by McClendon with no success. If the roster of such forms be made from the experience of investigators, I have little doubt that a large number of such species will appear. It seems to me, therefore, that there is a problem involved which should attract attention and be attacked from several points of view—cytologically, physical-chemically, chemically and from the standpoint of environmental differences. I have attempted to follow out a few of these lines, with the success noted in the paragraphs above. Until we are able to explain, at least in a way, why several eggs are not amenable to the same reagents and methods that a great variety of others are, our conception of fertilization and of the rôle of the spermatozoön cannot be final. It is only by the record of failures and successes that any advance in the knowledge of the matter in question will be made; the method of trial and error is

⁵ Tennent ('10) found that *Toxopneustes* × *Holothuria* went only to segmentation.

signally applicable here. The recent paper of Ralph Lillie ('12) is suggestive of a manner of approaching the problem from a slightly different point of view.

3. *Experiments with sperm extracts*

The experiments described here concern two things: (1) The repetition of Winkler's ('00) experiments with sperm extracts and (2) the effect of lecithin upon the unfertilized egg. It will be recalled that Piéri ('99) described experiments in which water extracts of spermatozoa were made by simple shaking with sea-water and filtering through filter-paper. Of course the experiment is fruitless because the spermatozoa pass readily through the paper. Winkler's experiments were more carefully performed, for he used distilled water as a medium for extracting the supposed 'ferment' although of course the use of distilled water, with a decidedly different osmotic pressure from the sea-water, introduces a variable wholly apart from any under control which he attempted to obviate by using salt solutions to restore the changed osmotic pressure. He found that when the extract which gave satisfactory results in the cold, is heated to 50°-60°C., no segmentation of the eggs results. Such a temperature would not permit a conclusion as to whether there is an enzyme present in the spermatozoon which affects the egg, for such temperatures are fatal to enzymes of all kinds (Bayliss '08, p. 12) as well as sperm.

For this reason, I repeated Winkler's work upon both *Cerebratulus* and *Arbacia*, by using only that temperature which killed the spermatozoa but no higher, which I found to be in the neighborhood of 40°C. This is a degree of heat which is seldom if ever fatal to enzymes and the optimum for many is but a few degrees below (37.5°C). Consequently, by bringing the spermatozoa in their fluid to this temperature in a test-tube and then cooling to 17°C, there is every reason to believe that no enzyme action has been affected, although of course positive proof is wanting. At any rate, in both *Cerebratulus* and *Arbacia*, no development in the eggs was observed, although a great variety of concentrations and dilutions were made in both cases. It has been suggested that Winkler's work was really a study in hypertonic solutions,

the distilled water extract being made up with boiled sea-water whose concentration was not determined, but which undoubtedly was hypertonic with sea-water. Hypertonicity indeed may be operative in the eggs of the two forms which I have examined, for the concentrated sperm is probably hypertonic with respect to sea-water, but in any case, it is not a factor of any consequence in either *Cerebratulus* or *Arbacia*.

Extracts of semen of *Arbacia* have been studied exhaustively by Gies ('01) and it would be useless to repeat these well-directed experiments except upon a different form. However, while Gies examined alcoholic and ether extracts, which contained the phospholipide, lecithin, this compound was not isolated so that its specific effects could be studied in case any existed. There seems to be a superstition hanging over lecithin⁶ as far as the physiology of this compound is concerned, for it has been assumed to be a growth-incident (Danilewski, Desgrey and Zaky), in sex-determination (Russo) and variously in therapeutics. Goldfarb has carefully examined the effect of lecithin obtained from hen's eggs and sheep's brains by the Roaf and Edie method, which is similar to Erlandsen's cold ether-aceton method, upon eggs of *Arbacia*, the eggs first having been fertilized with their own sperm. He found no evidence of acceleration in growth, that is, in the sequence of segmentation stages.

In my experiments, there were two sources of the lecithin used, one being hen's yolk⁷ by the Hoppe-Seyler method and the other ovaries and testes of *Arbacia*, extracted according to the following method: The organs of *Arbacia*, were shaken with a small amount of sea-water and the mass evaporated down on the water-bath

⁶ The lecithins, for there are different compounds with this designation, are esters of fatty acids with phosphorus and nitrogen. The term "lipoid" which adheres to them since Overton's christening, is wholly independent of their chemical relations, for what he meant by "fat-like bodies," concerned mainly the manner of obtaining them from their solutions, methods similar to the ones used in extracting fats, namely, by ether, alcohol, benzol, etc. In saponification, glycerophosphoric acid, cholin and the constituent fatty acid (palmitic, oleic, stearic, the special set of "lecithin" being designated according to the organic acid composing it), are formed.

⁷ I am indebted to Dr. Shiro Tashiro of the University of Chicago for the use of a portion of the preparation of lecithin from hen's yolk which he was employing in another set of experiments.

under slow heat to small proportions. The whole was then treated with cold ether for twenty-four hours in a flask, filtered, the filtrate treated with acetone in a separating funnel, the precipitate drawn off and filtered through paper. In the meantime, the residue from the first filtration was wrapped in heavy filter paper and extracted in a Soxhlet with ether and the treatment thereafter was similar to that of the second amount. The precipitate from the acetone was purified with absolute alcohol. A good deal of difficulty was experienced in getting rid of the pigments and at last this was given up, for it did not seem that luteins could influence the results in the minute quantities in which they appeared. By this method—that is, cold ether and hot ether together—one may obtain a large proportion of the lecithins present in the egg. The objection is that the use of hot ether may break the lecithins down into stearin, or whatever fatty acid is present, and in the experiments upon the eggs, it may be this which gives the effect in case any effect is observed. However, in neither the lecithin from the hen's yolk nor that from the gonads of *Arbacia*, did any positive evidence appear that the reagent exerted any effect whatsoever upon the unfertilized eggs. For this reason, no other method for obtaining lecithin was utilized.⁸

I am unable to convince myself that the final word has been said with respect to this matter. There is doubtless some key to the production of development in the unfertilized egg of such forms as we have mentioned which are refractory to artificial stimulation. Perhaps I have used too weak and perhaps too strong stimuli, although it seems that I should have discovered somewhere amongst the various strengths and various reagents, some combination that would have led to favorable results. It may be, as Lillie found in the star-fish, that the eggs become highly impermeable or are highly impermeable in the case of *Cerebratulus* to the reagents and that something must be done to render the permeability temporarily of lower value. But in saponin and other cytolytic reagents, it seems to me we find just such a reagent; and these I have used.

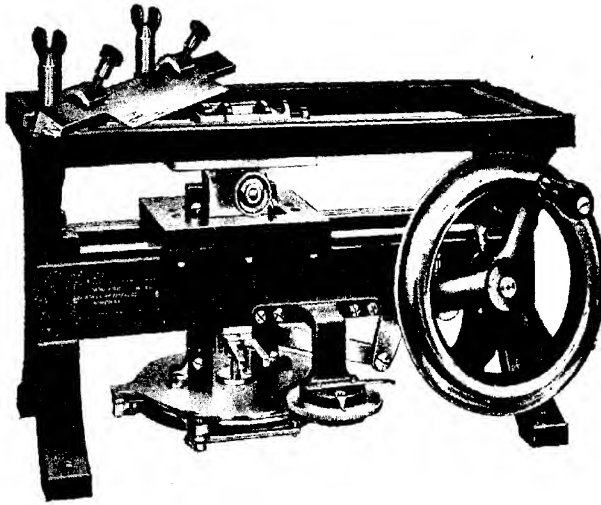
⁸ It is interesting to note that Robertson (*Journ. Biol. Chem.*, 12: 1) reports success with a product obtained by cytolyzing sea-urchin eggs extracting with water and finally precipitating with acetone, in inducing membrane formation.

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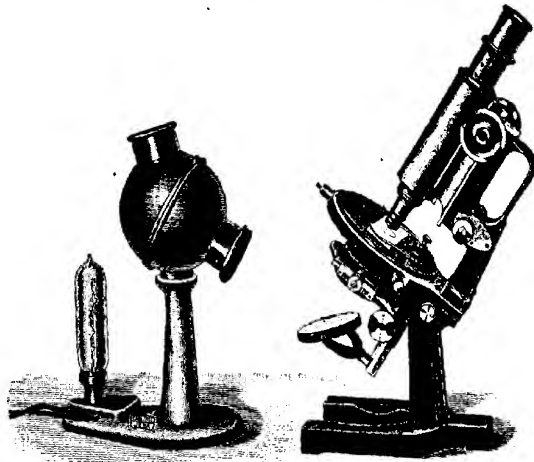
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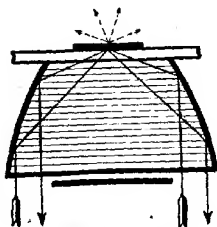
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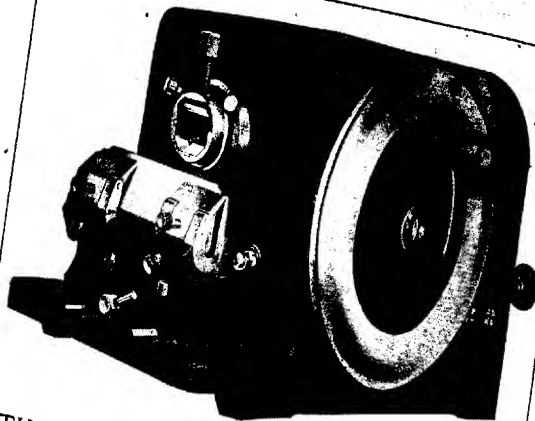
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INTRODUCTION: THE PRESENT STATE OF THE SUBJECT

The work, of which this article gives the results, has been directed toward throwing light on two main questions. The first of these may be roughly described as that of the 'osmotic properties' of vertebrate smooth muscle.¹ What are the results of subjecting smooth muscle to such experiments as are thought to demonstrate that the fibers of striated muscle are surrounded by semi-permeable membranes; and what are the physical conditions of the water, colloids, and crystalloids of the smooth muscle fibers? The second question is that of the chemical and physical processes which bring about contraction in smooth muscle. The close relation between these two questions will become apparent as the article progresses.

The investigations of the last fifty years have shown very clearly that the behavior of a tissue which is removed from the body and immersed in a foreign medium is often highly dependent on the osmotic pressure of that medium. It is a general rule that tissues remain longest in a state approaching the normal in those media which have about the same osmotic pressure as the blood plasma and lymph of the animal from which they were taken; they tend to give up fluid to hypertonic solutions and to take fluid from hypotonic solutions.¹ These facts have given rise to the view that the cells of animal tissues are surrounded by semi-permeable membranes and a great deal of work has been directed toward discovering the exact nature of these membranes.

Recently, however, the behavior of such colloids as gelatin and fibrin in various electrolytic and non-electrolytic solutions has received careful attention. It has been shown that these colloids have a remarkable tendency to take up water from certain solutions, and that the strength of this tendency depends in a complicated manner on the nature and concentration of the electrolytes present. It has been pointed out that the behavior of muscle under many conditions resembles that of gelatin and fibrin, and this resemblance has led certain investigators to deny the exist-

¹ The word 'hypertonic' is used throughout this article to mean that a solution has a higher osmotic pressure than the blood plasma of the animal under discussion; the word 'hypotonic,' to mean that the solution has a lower osmotic pressure than the blood plasma.

ence of semi-permeable membranes surrounding the animal cells. These investigators believe that the taking up and loss of water by muscle in various solutions is to be explained by a comparison of the behavior of muscle with that of gelatin and fibrin under similar conditions.

The osmotic properties of striated muscle

Overton² has made a thoroughgoing study of this whole question, using frog's striated muscle as the material for his experiments. He comes to the conclusion that the muscle fibers are surrounded by semi-permeable membranes of a peculiar nature — membranes permeable to water and to most fat solvents, but impermeable to sugars and inorganic salts. These membranes are, however, easily injured or destroyed; their destruction marks the irretrievable loss of irritability by the muscle; and after their destruction the tissue swells or loses water in various solutions somewhat as do masses of gelatin and fibrin.

The reasons which have led Overton and other investigators to believe that the striated muscle fibers are surrounded by semi-permeable membranes may be briefly summarized as follows:

The ash of striated muscle is entirely different from that of the blood plasma. It is well known that the blood plasma contains considerable amounts of diffusible NaCl, while the work of Katz³ indicates that the striated muscle fibers often contain little or none of this salt and considerable quantities of the phosphates of potassium. It is difficult to see how the salts of the blood plasma and of the muscle are prevented from interdiffusing unless there is some resistance to their passage at the surfaces of the muscle fibers.

The living striated muscle reacts in general to isotonic, hypertonic and hypotonic solutions of salts and sugars as if its fibers were surrounded by membranes permeable to water, but not to salt and sugar in solution, and as if they contained a salt solution isotonic with the blood plasma. The muscle maintains its origi-

² Overton; *Archiv für die gesammte Physiologie*, 1902, Bd. 92, pp. 115 and 346; 1904, Bd. 105, p. 207.

³ Katz; *Archiv für die gesammte Physiologie*, 1896, Bd. 63, p. 1.

nal weight in isotonic solutions of salts and sugars, gains weight in hypotonic solutions, and loses weight in hypertonic solutions.

The author has added some further evidence to that already given by Overton for the view that both osmosis and a process which may be called 'colloid swelling' play a part in the taking up of fluid by muscle from salt and sugar solutions.⁴ It may be shown that the behavior of frog's striated muscle when it is immersed in distilled water or in isotonic sugar solution depends on whether the tissue is 'living' or 'dead.' The living muscle swells in distilled water in such a manner as to suggest that osmosis plays a large part in the process; the water intake is large in the early stages and rapidly becomes less. The dead muscle, on the other hand, tends to take up water less rapidly in the early stages of its immersion than in the immediately succeeding ones. The living muscle maintains its original weight for many hours in an isotonic sugar solution while the dead muscle swells rapidly in such a solution.

Beutner has recently been able to throw still further light on this subject. He has shown that the behavior of muscle immersed in mixtures of salts with acids depends at first on the osmotic pressure of the mixtures, while it later bears little or no relation to osmotic pressure.⁵

If it be granted that the striated muscle fibers are surrounded by membranes which are more permeable to water than to salts and sugars in solution, a number of very important questions regarding the nature of these membranes and regarding the conditions which obtain within the muscle fibers remain to be answered. The first of these questions is whether the membranes are absolutely impermeable to salts and sugars, or whether they must be regarded only as offering a considerable resistance to their passage.

So far as I am aware, no one has definitely advocated the view that the muscle membranes are absolutely impermeable to salts and sugars.⁶ All the evidence that exists on the subject at pres-

⁴ Meigs; *American Jour. Physiol.*, 1910, vol. 26, p. 191.

⁵ Beutner; *Biochemische Zeitschrift*, 1912, Bd. 39, p. 280.

⁶ For Overton's opinion on this subject, see *Archiv für die gesammte Physiologie*, 1902. Bd. 92, pp. 382 and 383.

ent points to the view that potassium and phosphorus easily escape from the living muscle fibers, and that this loss is repaired by the entrance of these two substances into the fibers in some combination or combinations other than potassium phosphate.⁷ It is not doubted by biological chemists at present that the striated muscle fibers contain considerable quantities of phosphorus in organic combination.

The question how the muscle fibers are kept free or nearly free of sodium and chlorine cannot profitably be discussed at present, as there is no existing experimental evidence on the subject. It seems probable, however, that chemical as well as physical factors play a part in the maintenance of this condition also.

Overton has explained some of the facts observed by him by supposing that a certain portion of the water within the muscle fibers is held in chemical combination by the colloids as *organic water*, and does not act as a solvent for the muscle salts.⁸ He does not, however, give a detailed consideration to this part of the subject, leaving untouched the question whether the amount of the organic water is variable or constant. A good many facts could be explained by supposing the quantity of the organic water to be variable.

Schwarz has recently reported a number of experiments which indicate that any influence that causes a muscle to produce lactic acid tends to make it take up fluid from an isotonic salt solution.⁹ It seems improbable that the muscle's increased tendency to take up water is the direct result of the increase in the osmotic pressure of the muscle contents brought about by the presence of the lactic acid. In the first place, the osmotic pressure of such quantities of lactic acid as could be produced under the conditions of Schwarz's experiments would be infinitesimally small in comparison to the osmotic pressure of the muscle salts; and in the second place, Overton has found that the muscle membranes are quite permeable to lactic acid.¹⁰ An alternative explanation would be that the acid acts to make the muscle colloids combine

⁷ See Meigs and Ryan, *Jour. Biol. Chemistry*, 1912, vol. 11, pp. 409 and 410.

⁸ Overton; *Archiv für gesammte Physiologie*, 1902, Bd. 92, pp. 128-142.

⁹ Schwarz; *Biochemische Zeitschrift*, 1911, Bd. 37, p. 34.

¹⁰ Overton; *Archiv für die gesammte Physiologie*, 1902, Bd. 92, p. 267.

with more water, thus rendering the salts more concentrated in the remaining uncombined water.

The existing evidence, then, justifies the following conclusions in regard to frog's striated muscle: The surfaces of the muscle fibers are highly permeable to water, and offer a marked, though not absolute, resistance to the passage of salts and sugars in solution. The muscle is able to maintain a salt content different from that of the blood plasma by means of chemical processes which are not yet understood. A part of the water within the muscle fibers is held by the colloids as organic water, and does not act as a solvent for the muscle salts; and the taking up or loss of water by muscle immersed in isotonic and non-isotonic solutions is probably influenced by changes in the amount of the organic water within the fibers.

The osmotic properties of smooth muscle

In order to determine how far the 'osmotic properties' of smooth muscle resemble those of striated muscle, it will be necessary to know the results of subjecting smooth muscle to such experiments as have been carried out on striated muscle. The ash of smooth muscle has been analyzed by several investigators.¹¹ The results have shown that the smooth muscle fibers probably contain somewhat less potassium and phosphorus and somewhat more sodium and chlorine than the striated fibers of the same animal; but in most of the animals investigated the ash of the smooth muscle resembles that of striated muscle much more nearly than it does that of the blood plasma. In the case of the frog the smooth muscle fibers contain much more potassium and phosphorus and much less sodium and chlorine than does the blood plasma.¹²

These facts do not, however, show conclusively that the smooth muscle fibers are surrounded by semi-permeable membranes. In

¹¹ See Saiki, Jour. Biol. Chemistry, 1908, vol 4, p. 483; Costantino, Biochemische Zeitschrift, 1911, Bd. 37, p. 52; Macallum, Ergebnisse der Physiologie, 1911, p. 642; Meigs and Ryan, Jour. Biol. Chemistry, 1912, vol. 11, p. 401.

¹² For an analysis of the ash of frog's blood plasma, see Urano, Zeitschrift für Biologie, 1907, Bd. 50, pp. 218, 219, 224 and 225.

order to explain certain experimental results which have been obtained with striated muscle it has been necessary to suppose that this tissue contains potassium and phosphorus in combinations other than potassium phosphate and that a considerable part of the water of the tissue exists as organic water, which does not act as a solvent for salts. It has been pointed out by Meigs and Ryan that the conditions in smooth muscle could be explained without invoking the aid of semi-permeable membranes by applying to it the same suppositions in a somewhat extended form.¹³

It will be interesting, therefore, to have further evidence on the question whether the smooth muscle fibers are surrounded by semi-permeable membranes, and a large part of this article will be devoted to the presentation of such evidence.

The chemistry and physics of the contraction of smooth muscle

The author has already given evidence to show that the contraction of smooth muscle is the mechanical result of loss of fluid by its fibers.¹⁴ It has been found that the changes of weight which occur in smooth muscle as the result of its immersion in isotonic and non-isotonic solutions are seldom unaccompanied by corresponding changes in the length of the muscle fibers. These changes in length have been followed and recorded in the same experiments in which the weight changes undergone by the tissue have been studied, and they will be described and discussed in the proper place. It may be said here, however, that it is a very general rule that increase in the weight of the muscle is accompanied by lengthening; and decrease in weight by shortening; and this fact confirms the view that the contraction of the tissue is under normal circumstances the mechanical result of loss of fluid by its fibers. Certain experiments will later be described which suggest that the formation of lactic acid may play an important part in the physiology of the contraction of smooth muscle.

¹³ Loc. cit., pp. 411-413.

¹⁴ Meigs; American Jour. Physiol., 1908, vol. 22, p. 477; 1912, vol. 29, p. 317.

METHODS OF EXPERIMENTATION

It has been found convenient to put the protocols of the experiments together at the end of the article. These protocols give, for the most part, the results of experiments in which pieces of frog's muscle were immersed in various solutions and weighed at intervals. All the tissue came from three American species of frogs—the bull-frog (*Rana catesbiana*), the leopard frog (*Rana pipiens*), and the green frog (*Rana clamitans*). In most of the experiments the changes of length undergone by the smooth muscle were followed and the irritability of both the smooth and striated muscle used was tested at intervals. The temperature is also given with each experiment. In most cases this represents the temperature of the room at a point near that at which the experiment was carried out; it may be taken to be within a degree of that of the solution in which the muscle was immersed. In a few cases, where the effects of temperature were particularly to be studied, the actual temperature of the fluid in which the muscle was immersed is given.

The protocols represent selected experiments, which have been confirmed by a varying number of other unpublished experiments. I have not thought it worth while to add to the already rather formidable mass of material by publishing all the duplicate experiments. Duplicate experiments have been published in a few cases for the purpose of showing small variations in the behavior of the tissues, or because the points illustrated were thought to be particularly important.

The solutions used were made with water distilled over glass and with either Merck's or Kahlbaum's chemically pure compounds. The lactose used in Experiments 78 and 79 was a Merck preparation recrystallized several times and experimentally determined to be free from nitrogen and ash. It is difficult to get a pure lactose preparation, and smooth muscle behaves very differently in pure sugar solutions and in sugar solutions to which even very small quantities of electrolytes have been added. The Ringer solution used had the following formula:

NaCl.....	grams
KCl.....	0.65
CaCl ₂	0.02
NaHCO ₃	0.025
Distilled water.....	0.02
	100.00

The solutions generally were made up according to the method of Raoult; a 7.5 per cent saccharose solution, for instance, means a solution made by adding 7.5 grams of saccharose to 100 cc. of distilled water. The percentages of the solid constituents of the solutions are always given in terms of the anhydrous substances.

The sartorius has been selected as the example of striated muscle. This muscle can be easily dissected out without injury to any of its fibers, and the relations between its surface and volume are about the same as in the pieces of smooth muscle to be subsequently described. A small piece of connective tissue was always left attached to the pelvic end, and the tendon with a small chip of the tibia to the knee end. The preparation was handled through this chip of bone.

Most of the experiments with striated muscle are repetitions of experiments which have been already carried out by Overton. Those instances in which this is not the case will be particularly spoken of in the text. It has been thought well to publish the experiments on striated muscle, partly because Overton's work has been questioned in some quarters, and partly because it is often important to have a comparison between the smooth and striated muscle of the same individual frog.

The smooth muscle used in the experiments was obtained from the stomach. This organ was cut open along the line of the lesser curvature, and the mucous membrane was then torn loose from the muscular coat. The sheet of muscle so obtained usually weighed about twice as much as the sartorius from the same frog, but it was somewhat thinner so that the relations between its surface and volume were about the same.

Histological examinations of frog's sartorii fixed in various ways indicates that the muscle fibers make up about 75 per cent of their volume. The rest of the preparation consists of connective tissue and of the interstitial spaces between the fibers. The

work of Fahr¹⁵ on the chemical constituents of the ash of frog's striated muscle fibers confirms the view obtained by histological examination.

Histological examination of the preparations of smooth muscle described above indicates that the muscle fibers occupy about 80 per cent of the volume of such preparations, the remainder being made up of connective tissue and of interstitial spaces as in the case of striated muscle. Thin cross sections of living as well as of fixed smooth muscle may be examined histologically, and the proportional volume occupied by the fibers is about the same in both the living and fixed preparations.

It will be noticed that the striated muscle is prepared without cutting across any of its fibers, while the fibers of the smooth muscle preparations are cut across. A careful study of the effects of cutting across the fibers of striated and smooth muscle has been made.

Rigor very quickly sets in in the neighborhood of a cut across the fibers of striated muscle. If a frog's sartorius be cut across its middle, and the two pieces be immersed in Ringer's solution, whitish thickened areas make their appearance at the cut ends in the course of a minute or so. These areas increase gradually in size, so that at the end of perhaps four hours, the whole muscle is shortened, opaque and unirritable. Another uninjured sartorius used as a control may remain irritable for forty-eight hours in the same solution at the same temperature.

Nothing of this sort occurs as a result of cutting across the fibers of smooth muscle. I have cut sheets of smooth muscle across in several places in such a way that the fibers had a length of only 5 mm. between cuts. Such cut pieces of muscle have been kept in Ringer's solution at about 20°, ¹⁶ and their condition has been compared with that of other pieces of muscle in which the fibers had a length of 15 mm. between cuts. Both the controls and the cut pieces of muscle often remained irritable for forty-eight hours; the cutting made no difference whatever in the length of time which the tissue remained irritable, and there

¹⁵ Fahr: *Zeitschrift für Biologie*, 1908, Bd. 52, p. 80.

¹⁶ All temperatures are given in terms of the centigrade scale.

was never anything to indicate that the smooth muscle went into rigor either in the neighborhood of the cuts or in any other part. It may even be shown that cross sections of frog's stomach muscle cut off with a sharp razor and only 0.1 to 0.2 mm. thick remain irritable for several hours in Ringer's solution at room temperature. Such slices of muscle may be seen to contract when stimulated beneath the microscope after they have been for several hours in Ringer's solution at 20°.

Other experiments have been carried out with the object of determining the effect which cutting across the fibers of striated and smooth muscle has on the changes of weight undergone by the two tissues in Ringer's solution. It has been found that cutting the fibers of striated muscle causes the tissue to gain weight in Ringer's solution. In the case of smooth muscle, the behavior of preparations in which the fibers have been cut across in only one place has been compared with that of others in which several cuts have been made; and it has been found that the latter have, if anything, a slightly less tendency to gain weight than the former.

It is clear, therefore, that cutting across the fibers of smooth muscle has no demonstrable injurious effect on them; and the results of the experiments to be subsequently described will show that the differences in the behavior of striated and smooth muscle cannot be attributed to the fact that the fibers of the latter tissue have been cut across.

Experiments, in which the changes of weight undergone by muscle in various solutions have been determined, have now been carried out by so many investigators that it is unnecessary to give a detailed defense of the accuracy of such experiments. It is, of course, to be understood that the object is always to follow accurately the change of weight undergone by a piece of tissue, and not to get, at any time, its absolute weight, which is a still undefined quantity.

Overton states¹⁷ that in his experiments on *sartorii* the limits of error were within 3 mg. In general I have followed the technique which he has described, but I am convinced that the limits

¹⁷ Overton; *Archiv für die gesammte Physiologie*, 1902, Bd. 92, p. 126.

of error in my experiments with sartorii are less than those which he gives, perhaps within 1 mg. The chief source of error is, of course, the drying of the tissue on filter paper which is necessary before weighing; unless the tissue is dried to the same extent each time, the results do not truly represent the course of gain or loss of weight. After a certain amount of practice one learns to keep to a very uniform routine in the matter of drying, and to recognize immediately the errors which sometimes occur when one is fatigued. Experiments in which errors have occurred must of course be discarded. I may say, however, that I have been obliged to discard only very few experiments on this account; the general character and smoothness of the curves of striated muscle are sufficient evidence for the view that the errors in weighing have been small.

In the case of the smooth muscle the drying is not quite so easy as with the striated muscle, for the sheets of tissue often exhibit a considerable tendency to curl up. One soon learns, however, to flatten them out on the filter paper in a uniform manner; the limits of error here are certainly within 2 mg.

The irritability of the tissues was tested by means of a strong interrupted Faradic current. I determined whether the striated muscle responded to the stimulus or not by direct inspection. In the case of the smooth muscle, strips of the tissue were attached to a light lever which magnified about seven times and the point of this was brought against a scale. In this way very slight changes in length could be determined. A considerable amount of experience with the effects of various kinds of stimuli on smooth muscle has convinced me that a strong rapidly interrupted Faradic current is by far the most satisfactory, if one merely wishes to determine in a rough way the state of the tissue's irritability. I have never failed with this stimulus to produce responses in fresh tissue, and the responses are fairly constant in size and rapidity. I have, of course, been careful to keep my current far below the strength which would produce heat shortening in dead tissue.

In the case of striated muscle the irritability of the actual muscle weighed was determined. This could be done with almost no manipulation of the muscle; and as the application of the current

and the resulting contraction lasted only a fraction of a second, it may be supposed that the chemical change produced had a negligible effect on the course of the tissue's changes in weight. A good deal more manipulation was necessary to satisfactorily test the irritability of the preparations of smooth muscle. Where this did not have to be done until the end of the experiment, strips were cut off from the actual piece of muscle which had been weighed, and tested as described above: when it was desirable to test the irritability of the smooth muscle in the middle of an experiment, the piece of muscle used was cut into a strip and a larger sheet and the two portions were immersed in the solution; the strip was used for the testing of irritability and the larger sheet for weighing.

It will be noticed that it is reported at the end of some of the experiments that pieces of smooth muscle showed a tendency to lengthen on stimulation. This seemed to be the case after the tissue had remained for some time in various solutions, but particularly as the result of immersion in double strength Ringer solution. Subsequent experiments, in which the changes of length undergone by the tissue as the result of stimulation after a stay in double strength Ringer, were recorded on a kymograph instead of being studied as described above, showed that the stimulation usually produced a very small shortening followed by a comparatively large lengthening. In only a few cases did the preliminary shortening fail altogether to appear.

The results of some of the experiments are given in curves, which appear throughout the article. In these the points of weighing are prominently indicated by crosses.

Some of the experiments give the results of immersing connective tissue in various solutions. The connective tissue was obtained from the tendo Achillis of large frogs, and the technique was the same as in the experiments on muscle.

A few of the experiments give the results of attempts to determine directly by chemical analysis to what extent sodium and potassium diffuse out of smooth muscle into a surrounding isotonic sugar solution, and to what extent the sugar diffuses into the muscle. These experiments require no particular comment in this place.

A COMPARISON OF THE CHANGES OF WEIGHT UNDERGONE BY
SMOOTH AND STRIATED MUSCLE IN VARIOUS ISOTONIC
AND NON-ISOTONIC SOLUTIONS

It will be shown in this section that the changes of weight undergone by smooth muscle in various solutions of electrolytes and non-electrolytes are quite different from those undergone by striated muscle under similar circumstances. The changes of weight undergone by the smooth muscle bear only a rough relation to the osmotic pressure of the solution in which it is immersed, and are such as to make it difficult to believe that any considerable portions of the tissue are separated from their surroundings by semi-permeable membranes. But a discussion of the bearing of the results will be reserved until later.

Experiments with Ringer's solution. Both the smooth and striated muscle of the frogs with which I have worked maintain their irritability, as a rule, for forty-eight hours or more in Ringer's solution at 20°. The sartorii vary somewhat in regard to the changes of weight which they undergo. They sometimes maintain their original weight for many hours, sometimes gain in weight, and sometimes lose. The gain or loss in weight is, however, not often more than 10 per cent of the original weight of the muscle in the course of twenty-four hours, and the Ringer solution may be considered to be on the average isotonic—that is, about as many sartorii tend to gain as to lose weight in it.

The stomach muscle of the same frogs practically always gains weight when immersed in Ringer's solution. The amount of this gain is variable being sometimes less than 10 per cent and sometimes over 30 per cent. If the changes of weight undergone by the stomach muscle and sartorius from the same frog be compared with each other, it will be found that in those cases where the sartorius tends to lose weight the stomach muscle gains little; and in those cases where the sartorius gains, the stomach muscle gains much more. In other words, the stomach muscle has a decidedly greater tendency to take up fluid from Ringer's solution than has the sartorius. Figure 1 gives the curves of change in weight of the stomach muscle and sartorius from the same frog in a typical experiment.

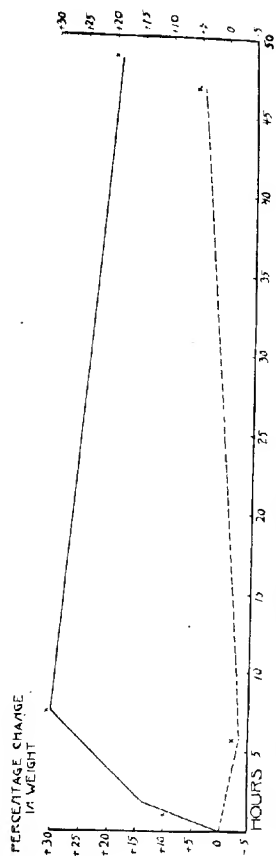


Fig. 1 Changes in weight undergone by the sartorius (broken line) and stomach muscle (unbroken line) of a frog in Ringer's solution. See Experiments 8 and 9.

A number of experiments have been carried out with the view of determining the factors which cause the tissues of some frogs to take up more fluid from Ringer's solution than those of others. It has been found to be a general rule that the tissues of the larger frogs of a species tend to take up more fluid than those of the smaller ones. This rule has nothing to do with the actual size of the tissues used. A 'small' bull-frog may be larger than a 'large' leopard frog, but the stomach muscle and sartorius of the former tend to take up less fluid from Ringer's solution than those of the latter. The rule is of interest in connection with the now well known fact that the tissues of the older (and larger) members of a species tend to contain more solids and less water than those of the younger members.¹⁸ Experiments 26, 27, 30 and 31 show the variations in the manner in which the tissues of the different sized frogs of a species behave in Ringer's solution.

There are a number of other factors besides the age and size of a frog which have an influence on the amount of fluid which its stomach muscle will take up from Ringer's solution. The effects of temperature are, perhaps, as striking as those of any other influence. Figure 2 gives a comparison of the amount of fluid taken up from Ringer's solution by two pieces of muscle from the stomach of the same leopard frog which were kept at about 0.5°C. and at room temperature respectively. The piece of muscle kept at room temperature took up more than twice as much fluid as the other.

Experiments with diluted and concentrated Ringer's solution. Both the striated and smooth muscle of the frog remain alive for many hours in double strength Ringer and in half strength Ringer,¹⁹ and the changes of weight undergone by the two tissues when transferred from Ringer to one or the other of these solutions are very interesting. They add still further evidence to that which has just been presented for the view that the surfaces

¹⁸ See v. Bezold, *Zeitschrift für wissenschaftliche Zoologie*, 1857, Bd. 8, p. 487; and Donaldson, *Jour. Comp. Neur.*, 1910, vol. 20, p. 119.

¹⁹ By 'half strength Ringer' is meant a Ringer solution diluted with its own volume of distilled water, and by 'double strength Ringer' a solution in which all the salts have double the concentration that they have in ordinary Ringer.

of the smooth muscle fibers are quite permeable both to the muscle salts and to the salts of Ringer's solution.

Overton has pointed out²⁰ that the behavior of muscle in non-isotonic solutions may better be studied after the tissue has been kept for some time in a nearly isotonic solution. It will be seen that this procedure presents difficulties in a comparison of smooth with striated muscle; the two tissues from the same frog hardly ever maintain the same weight in any salt solution. I have, however, overcome this difficulty as far as possible by using the tissues both of those frogs of which the smooth muscle swells markedly in Ringer and of those of which it does not. There is no important difference in the results obtained in the two cases.

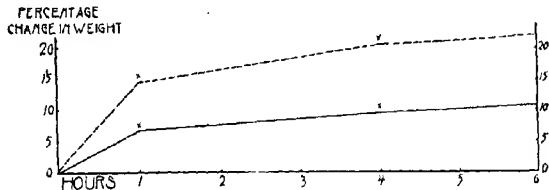


Fig. 2 Changes in weight undergone by two pieces of the stomach muscle of a frog, of which one (broken line) was immersed in Ringer's solution at between 20° and 21°, and the other (unbroken line) in Ringer's solutions at between 0° and 1°. See Experiments 19 and 20.

Smooth muscle has another peculiarity which must be taken account of in the experiments to be described. If a piece of the tissue be placed in Ringer and weighed at long intervals, say every half hour, it will be found that its change in weight takes a certain line. But, if at the end of an hour or so the weighings be suddenly increased in frequency to once in five or ten minutes, the tissue may begin to lose weight rapidly and continue to do so through several weighings. The same thing is true to a very much less extent for striated muscle. Figure 3 shows the difference in the effects of increasing the frequency of weighings and dryings in the two tissues.

²⁰ Overton; *Archiv für die gesammte Physiologie*, 1902, Bd. 92, p. 128.

This difficulty was overcome, as far as possible, by leaving the two tissues first for a considerable time in Ringer, and transferring them to 50 per cent Ringer only after they had undergone a number of weighings in Ringer at the same intervals as were subsequently to be used in the 50 per cent Ringer. Figure 3 shows the results of an experiment in which, as it happened, the two kinds of muscle, under the influence of the frequent weighings in Ringer, reached a constant weight which in both cases was between 7 and 8 per cent less than their original weight.

As figure 3 shows, the curves of swelling of the two tissues in 50 per cent Ringer are quite different from each other. During the first eight minutes after transfer to the hypotonic solution the striated muscle swells five times as rapidly as the smooth muscle; the swelling curve of the former is more or less exponential in character while that of the latter tends toward being a straight line.

I have carried out a number of experiments of this sort in which the detail has been much varied. The muscles have come from large and small individuals of leopard, green and bull-frogs. Sometimes the piece of stomach has been larger and sometimes the sartorius. The surface exposed by the two pieces in relation to the volume has not differed very widely, but was probably greater in the case of the stomach muscle. This ought to have caused a more rapid early swelling in the case of the stomach muscle, if other conditions had been the same. In one case the swelling curve of a sartorius in 50 per cent Ringer was studied after its fibers had been cut across. The results did not differ materially from those obtained with the uncut sartorii; for, though cutting across the fibers of striated muscle causes it to swell slowly in Ringer's solution, this swelling is too slow to markedly alter the course of swelling in the first half hour after transference from Ringer to 50 per cent Ringer.

In all these experiments the results were essentially the same—a fairly regular curve for the striated muscle rising rapidly in the early stages, and a more irregular curve for the smooth muscle much slower in the early stages and tending to be a straight line.

Figure 4 shows the results of an experiment in general similar to that shown in figure 3.

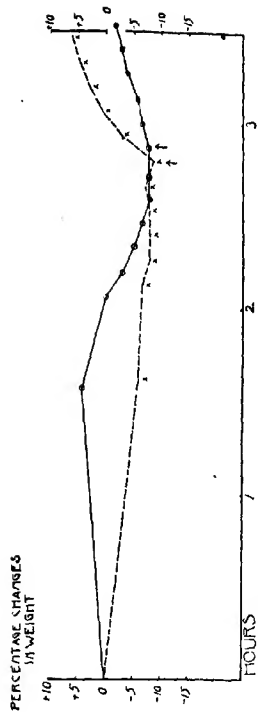


Fig. 3 Changes in weight undergone by the sartorius (broken line) and stomach muscle (unbroken line) of a frog immersed first in Ringer and then (arrows) in half strength Ringer. See Experiments 32 and 33.

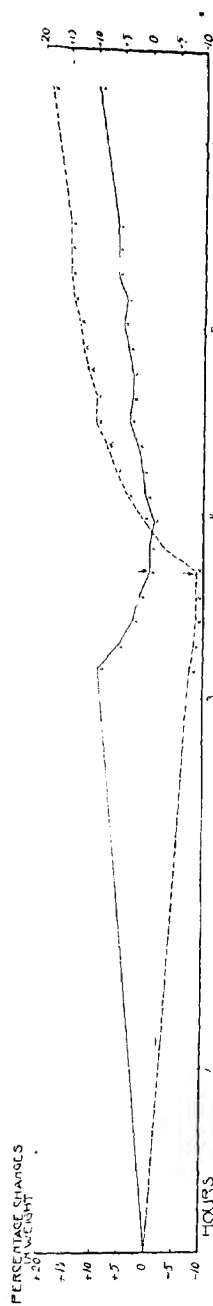


Fig. 4 Changes in weight undergone by the sartorius (broken line) and by the stomach muscle (unbroken line) of a frog. The tissues were immersed first in Ringer, and then (arrows) in 50 per cent Ringer. See experiments 36 and 37.

I have carried out a number of experiments in which the procedure was exactly the same as in those which have just been described, except that at a certain point in the experiment the tissue was transferred from Ringer to double strength Ringer instead of to 50 per cent Ringer. Figures 5 and 6 give the results of two such experiments. If figure 6 be compared with figure 4, it will be seen that the striated muscle loses weight in the hypertonic Ringer very much as it gains weight in the hypotonic Ringer. The curves in both cases are at first roughly exponential and then tend to be straight lines, and the rapidity of loss in the double strength Ringer is about equivalent to the rapidity of gain in the half strength Ringer. In the smooth muscle the results are widely different. The curves here show no indication of definite mathematical character; the loss of weight during the second interval after transference to the hypertonic solution is more rapid than during the first interval. Perhaps, however, the most surprising result is the difference between the rapidity of the loss undergone by the smooth muscle in the hypertonic solution and that of the gain undergone by the same tissue in the hypotonic solution. In the first sixteen minutes after transfer to the hypotonic solution, the tissue gains only 2 per cent of its original weight while in the first sixteen minutes after transfer to the hypertonic solution it loses 15 per cent. The same result has been obtained in most of the experiments which I have carried out.

Experiments with solutions of single electrolytes. Overton and other investigators have carried out experiments in which the weight changes of frog' striated muscle in solutions of various salts have been followed. These experiments have shown that as a general rule the tissue tends to maintain its original weight in solutions which have about the same osmotic pressure as a 0.7 per cent NaCl solution, to lose weight in solutions hypertonic to this, and to gain weight in hypotonic solutions. The theory that the striated muscle fibers are surrounded by semi-permeable membranes rests partly on the results of such experiments.

In 0.7 per cent NaCl solution smooth muscle gains in weight at first rather more rapidly than in Ringer's solution, but this

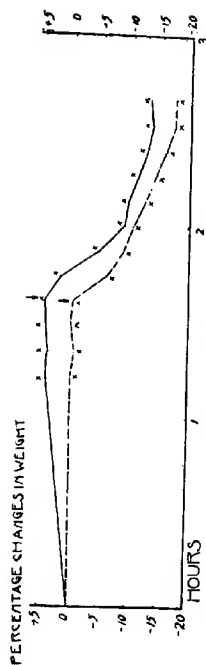


Fig. 5 Changes in weight undergone by the sartorius (broken line) and by the stomach muscle (unbroken line) of a frog. The tissues were immersed first in Ringer and then (arrows) in double strength Ringer. See Experiments 51 and 52.

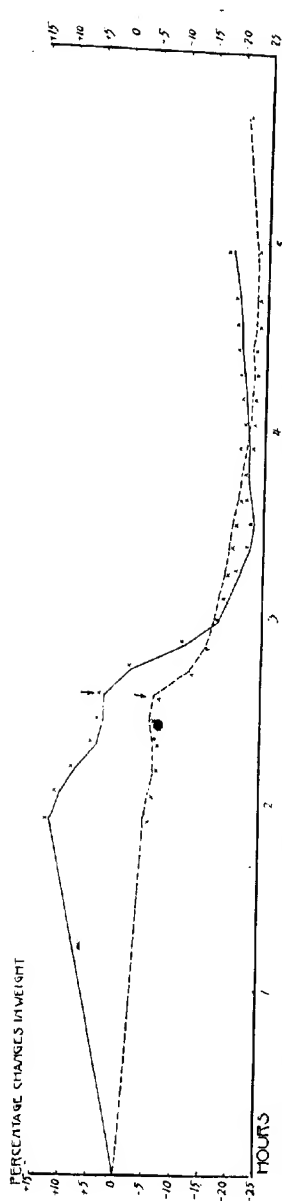


Fig. 6 Changes in weight undergone by the sartorius (broken line) and by the stomach muscle (unbroken line) of a frog. The tissues were immersed first in Ringer and then (arrows) in double strength Ringer. See Experiments 54 and 55.

gain soon comes to an end and is followed by a fairly rapid loss in weight (fig. 7 and Experiments 34, 35 and 36). The tissue may remain somewhat irritable for twenty-four hours in the NaCl solution.

Other experiments have been tried in which pieces of striated and smooth muscle were immersed in 0.9 per cent KCl solution, 1.3 per cent K_2HPO_4 solution, and 1 per cent $NaC_2H_3O_2$ solution, all of which have very nearly the same osmotic pressure as a 0.7 per cent NaCl solution (fig. 8 and Experiments 64, 65, 66, 67, 68, 69, 72 and 73). In the KCl solution the smooth muscle first loses weight and then gains from 45 to 55 per cent of its original weight, though it remains irritable through the twenty hours or more of the experiment. In the other solutions it gains slowly and steadily and to about the same extent as it does in Ringer's solution. It maintains its irritability for about twenty-four hours in these solutions also.

In connection with the question of the reactions of muscle to potassium chloride, certain facts must be mentioned, which have been overlooked by Overton, and which show in a striking way how dangerous it is to theorize about the nature of the semi-permeable membranes of striated muscle. Overton makes the claim that the surfaces of the living striated muscle fibers are impermeable to the salts of the alkalies and of the alkali earths and to their ions.²¹ The evidence for this view is that living striated muscle maintains its original weight in isotonic solutions of these salts. Overton knew, however, that striated muscle swells quite rapidly in isotonic solutions of certain salts, notably KCl, and he thought that the tissue was rapidly killed by an isotonic solution of this salt. He draws a distinction between KCl, KBr, KI and KNO_3 on the one hand and K_2HPO_4 , K_2SO_4 , $K_2C_4H_2O_4(OH)_2$, $KC_2H_3SO_4$ and $KC_2H_3O_2$ on the other.²² He maintains that in isotonic solutions of the former salts the muscle swells and is soon killed, while in isotonic solutions of the latter it does not swell and is only temporarily paralyzed and its irritability may be quickly restored by transferring it to Ringer's solution. In

²¹ Overton; *Archiv für die gesammte Physiologie*, 1904, Bd. 105, p. 281.

²² Overton; *Loc. cit.*, p. 199.

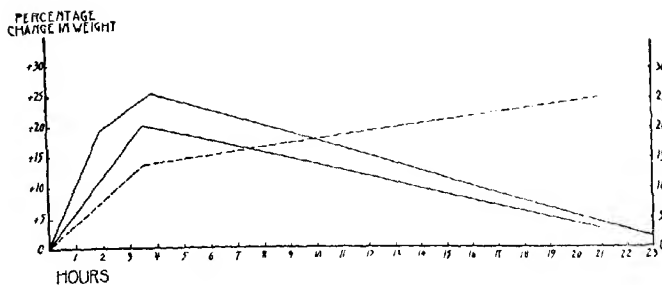


Fig. 7 Changes in weight undergone by three strips of living smooth muscle, of which one (broken line) was immersed in Ringer's solution; and the other two (unbroken lines) in 0.7 per cent NaCl solution. See Experiments 34, 35 and 36.

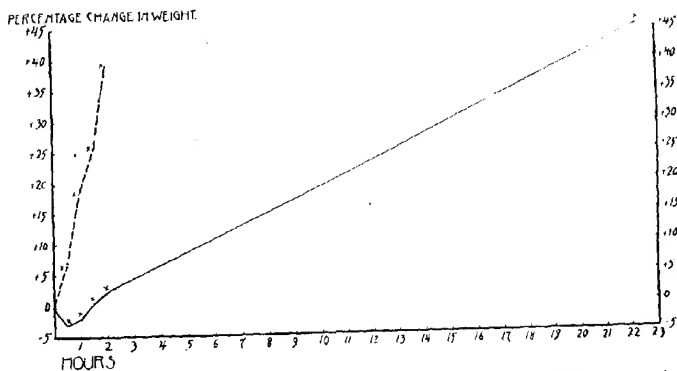


Fig. 8 Changes in weight undergone by the sartorius (broken line) and stomach muscle (unbroken line) of a frog in 0.9 per cent KCl solution. See Experiments 64 and 65.

experimenting with isotonic KCl solution I have found that it does not kill frog's striated muscle even in several hours at temperatures below 18°. It takes the muscle a good deal longer to recover in Ringer's solution after immersion in KCl than after immersion in K_2HPO_4 , but the recovery does take place in time very completely, even though the swelling in the KCl solution may have amounted to 50 per cent of the original weight of the muscle (Experiments 64, 66, 72, 74 and 77).

The membranes surrounding the fibers of striated muscle must, therefore, be regarded as quite permeable to KCl, and this would seem to make it difficult to generalize about their chemical nature. They must differ in an interesting way from the membranes surrounding the red blood cells, which are notably impermeable to KCl.²³ The permeability of the muscle membranes to KCl may play an important part in the maintenance by the fibers of their normal potassium content.

Experiments with solutions of non-electrolytes. One of the strongest reasons for believing that the fibers of frog's striated muscle are surrounded by semi-permeable membranes, is the fact that the tissue maintains its original weight for many hours in isotonic solutions of sugar, gains weight in hypotonic solutions, and loses weight in hypertonic solutions of this substance. Overton has tried the effect of immersing the tissue in isotonic solutions of various sugars and of a number of other non-electrolytes. He finds that it maintains its original weight in isotonic solutions of sugars and of amino acids,²⁴ but gains weight more or less rapidly in isotonic solutions of glycerine and urea.²⁵ He concludes that the muscle membranes are permeable to the last two substances and impermeable to the others.

I have followed the weight changes undergone by smooth muscle in isotonic solutions of cane sugar, lactose, dextrose and alanin. The tissue gains weight rapidly in all of these solutions (figs. 9,

²³ Hamburger; *Osmotischer Druck und Ionenlehre*, Wiesbaden, 1902, vol. 1, pp. 208 and 209.

²⁴ Overton; *Archiv für gesammte Physiologie*, 1902, Bd. 92, pp. 215, 224, 233; see also pp. 352-357.

²⁵ *Loc. cit.*, pp. 197, 198 and 205-207.

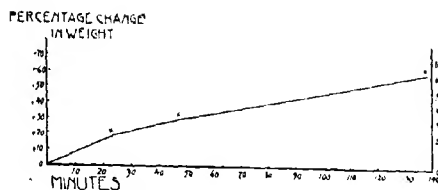


Fig. 9 Changes in weight undergone by living smooth muscle in 7.5 per cent cane sugar solution. See Experiment 15.

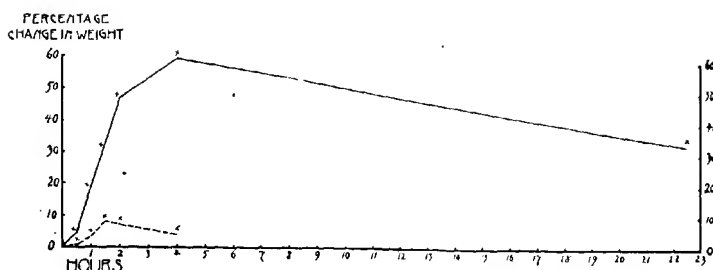


Fig. 10 Changes of weight undergone by the sartorius (broken line) and stomach muscle (unbroken line) of a frog in 7.5 per cent lactose solution. See Experiments 78 and 79.

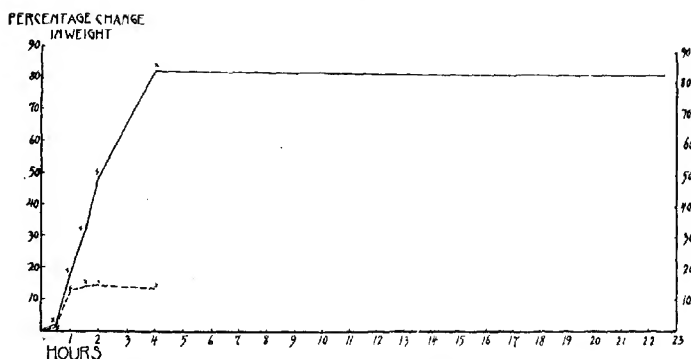


Fig. 11 Changes in weight undergone by the sartorius (broken line) and stomach muscle (unbroken line) of a frog in 3.95 per cent dextrose solution. See Experiment 75 and 76.

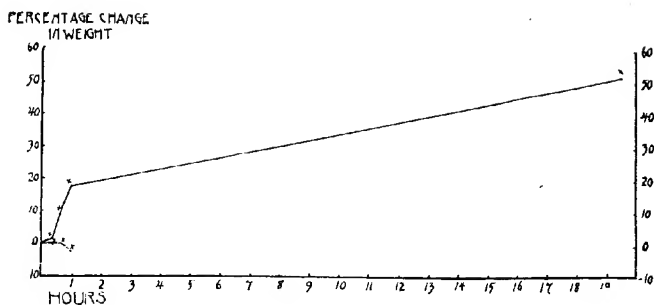


Fig. 12 Changes in weight undergone by the sartorius (broken line) and stomach muscle (unbroken line) of a frog in 2.3 per cent alanine solution. See Experiments 45 and 46.

10, 11 and 12 and Experiments 15, 25, 46, 76 and 79). Experiments 14, 45, 75 and 78 show the behavior of the striated muscle from the same frogs in the same solutions. The smooth muscle remains alive in these solutions decidedly longer than does the striated muscle.

I have also found that both smooth and striated muscle gain weight rapidly in isotonic solutions of glycerine and urea, but I have not thought it worth while to publish detailed accounts of the experiments with these substances, as the two tissues soon lose their irritability in solutions of them. The striated muscle goes into rigor, while the fibers of smooth muscle lengthen enormously. In a few cases preparations of striated and smooth muscle have been immersed in hypertonic solutions of dextrose and cane sugar (Experiments 21, 22, 49, 50, 70 and 71). The striated muscle tends to lose weight in such solutions, while the smooth muscle gains weight, though not quite so fast as in the isotonic solutions. The behavior of the smooth muscle in these sugar solutions is, however, rather capricious. If Experiment 50 be compared with Experiment 71, it will be noticed that the muscle in the stronger solution has gained a larger percentage of its original weight at the end of two hours than the muscle in the weaker solution.

These experiments were carried out before I was aware of the fact that the rapidity with which a piece of smooth muscle swells in a sugar solution depends very largely on the size of the piece of muscle and on the amount of the sugar solution with which it comes in contact. It will be shown later that a certain amount of sodium (in all probability combined with chlorine) diffuses out from smooth muscle immersed in an isotonic sugar solution; and very small amounts of sodium chloride added to an isotonic sugar solution greatly reduce or prevent altogether the swelling

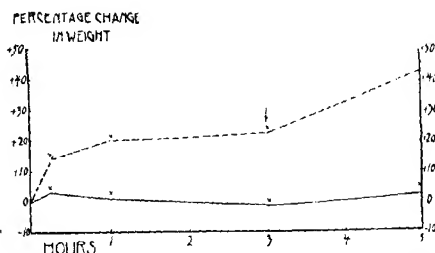


Fig. 13 Changes in weight undergone by two pieces of living smooth muscle, of which one (broken line) was immersed in 7.5 per cent cane sugar solution; and the other (unbroken line) in a mixture containing 19 parts of the sugar solution and 1 part Ringer. At the point marked with an arrow the pure sugar solution was changed. See Experiments 60 and 61.

of a piece of smooth muscle immersed in it. As evidence for this last statement figure 13 and Experiments 60 and 61 may be cited.

Experiments with distilled water. A comparison has been made of the behavior of striated and smooth muscle in distilled water (figs. 14, 15 and 16). The discussion of these results will be reserved until later.

Experiments with acidified Ringer's solution. It has been shown by various investigators that smooth muscle may contain small quantities of lactic acid.²⁶ It is now a well recognized fact that lactic acid plays an important part in the physiology of

²⁶ See Saiki; Jour. Biol. Chemistry, 1908, vol. 4, p. 485; Meigs, American Jour. Physiol., 1909, vol. 24, pp. 5 and 6.

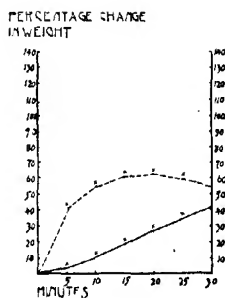


Fig. 14 Changes in weight undergone by a living sartorius (broken line) and by a dead sartorius (unbroken line) immersed in distilled water. See Experiments 2 and 4.

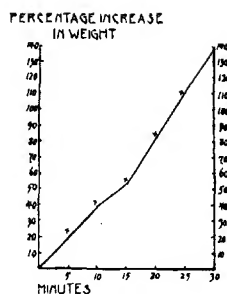


Fig. 15 Changes in weight undergone by smooth muscle immersed while still living in distilled water. See Experiment 3.

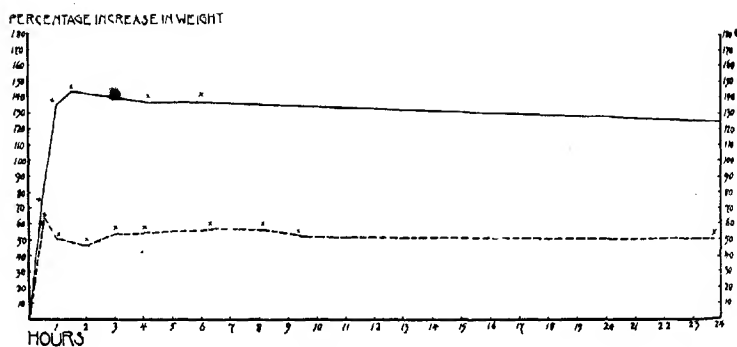


Fig. 16 Changes in weight undergone by frog's sartorius (broken line) and stomach muscle (unbroken line) immersed in distilled water. See Experiments 1 and 13.

striated muscle, and it is an interesting question what effect small quantities of acid would have on the tendency of the smooth muscle fibers to imbibe fluid.

A number of experiments have been carried out with the view of answering this question. It is, of course, not practicable to add the lactic acid to the Ringer's solution described on p. 505, for the NaHCO_3 contained in that solution would react with small quantities of the acid. For this reason the behavior of pieces of smooth muscle in Ringer's solution has been compared, on the one hand, with the behavior of pieces of muscle in a Ringer solution from which the NaHCO_3 has been omitted, and, on the other,

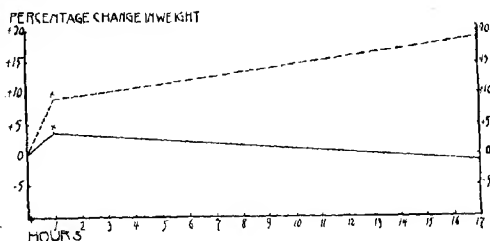


Fig. 17 Changes in weight undergone by two strips of living smooth muscle, of which one (broken line) was immersed in Ringer's solution; and the other (unbroken line), in a Ringer solution in which 0.01 per cent of lactic acid had been substituted for the NaHCO_3 . See Experiments 27 and 28.

with the behavior of pieces of muscle in a Ringer solution in which a small quantity of lactic acid has been substituted for the NaHCO_3 . The results have shown that similar pieces of muscle gain weight to about the same extent in Ringer's solution and in Ringer without NaHCO_3 ; but that the tendency to gain weight is almost absent in a Ringer solution in which 0.01 per cent of lactic acid is substituted for the NaHCO_3 , and is soon succeeded by a tendency to lose weight (fig. 17 and Experiments 10, 11, 16, 27, 28, 36, 37 and 53).

Pieces of smooth muscle tend to take up fluid from a Ringer solution in which larger quantities of lactic acid have been substituted for the NaHCO_3 (Experiments 23 and 24).

THE CHANGES OF WEIGHT UNDERGONE BY CONNECTIVE TISSUE
IN VARIOUS SOLUTIONS

The changes of weight undergone by connective tissue in certain solutions has been studied for the purpose of having the reactions of a third tissue to compare with those of striated and smooth muscle.

Connective tissue gains weight in Ringer's solution and to about the same extent as does smooth muscle. In three experiments in which bull-frogs' tendons were left for from seventeen to twenty-four hours in Ringer's solution at temperatures between 18° and 22°C. the average maximum gain in weight was 20.2 per cent, the smallest gain being 14.6 per cent and the largest 23.1 per cent. In figure 18 the curve of gain in weight of one of these pieces of tendon is compared with that which was obtained in one of the experiments on stomach muscle. The curve of gain in weight of the smooth muscle is somewhat more irregular than that of the tendon but has otherwise very much the same character. It is easy to understand that the swelling in the case of the smooth muscle might be more or less irregular when one remembers how marked an effect small quantities of lactic acid have on the swelling of this tissue. The smooth muscle remained highly irritable through the whole course of the experiment recorded in figure 18, and it is not at all an improbable supposition that the course of the swelling was modified by the production of metabolites by the muscle.

Temperature has an influence on the swelling of tendon in Ringer's solution more or less like that which it has in the case of smooth muscle. Figure 19 gives the swelling curves of two pieces of tendon from the same frog kept in Ringer's solution at room temperature and at between 0° and 1° respectively. It will be seen that the swelling of the tendon is considerably slowed by cold. If, however, figure 19 be compared with figure 2 which gives the results of a similar experiment on smooth muscle, it will be seen that the effect of cold on the swelling of tendon differs considerably from that which it has on the swelling of muscle. The effect of cold on the tendon is much less marked, and at

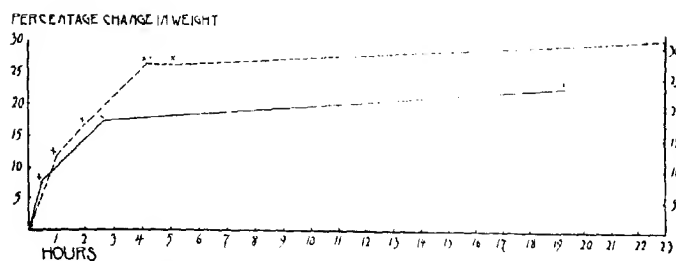


Fig. 18 Changes of weight undergone by smooth muscle (broken line) and by tendon (unbroken line) in Ringer's solution. See Experiments 18 and 38.

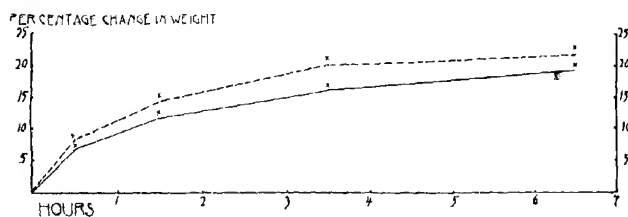


Fig. 19 Changes of weight undergone by the Achilles tendons of a bull-frog in Ringer's solution at between 20° and 22° (broken line) and at between 0° and 1° (unbroken line), respectively. See Experiments 47 and 48.

the end of two or three hours the tendon kept at the lower temperature begins to gain faster than the other. In the case of the smooth muscle, on the other hand, the swelling is slower at the lower temperature through the whole six hours of the experiment.

The swelling of tendon in 0.7 per cent NaCl solution does not differ much from that which takes place in Ringer's solution. Three experiments were carried out in which pieces of tendon were immersed for from nineteen to twenty-four hours in 0.7 per cent NaCl solution at temperatures varying between 18° and 21°. At the ends of the experiments the pieces of tissue weighed 14.5 per cent, 18.2 per cent and 20.4 per cent more than originally, the average gain being 18 per cent. The weight of the three pieces of tissue increased gradually throughout the course of the experiment; there was no tendency toward a rapid gain followed after three to six hours by a loss, as there is in the case of smooth muscle immersed in 0.7 per cent NaCl solution. Experiment 40 gives the details of the change of weight undergone by tendon in 0.7 per cent NaCl solution.

In 7.5 per cent cane sugar solution, tendon gains weight to about the same extent as in Ringer (Experiment 29). And in distilled water tendon gains weight very much less than does smooth muscle and not very markedly more than in Ringer's solution (Experiment 41).

Tendon swells less in Ringer without NaHCO_3 than in ordinary Ringer (Experiments 38, 42, 43 and 47). But if small quantities of lactic acid be added to the Ringer without NaHCO_3 , the tendon swells markedly more than in Ringer (Experiments 39 and 44).

SUMMARY OF THE RESULTS SO FAR DESCRIBED

In the preceding pages the changes of weight undergone by smooth muscle in various solutions has been compared, on the one hand, with the reactions of striated muscle, and on the other, with those of tendon. The results are in full accord with the prevalent view that the surfaces of the living striated muscle fibers are highly impermeable to salts and sugars. The results with smooth muscle and tendon, however, indicate that no considerable

portions of these tissues are surrounded by semi-permeable membranes. The changes of weight undergone by smooth muscle and tendon in various solutions bear only a rough relation to the osmotic pressures of the solutions, and are in all probability analogous to the changes of weight undergone by gelatin and fibrin under similar conditions. The differences in the behavior of smooth and striated muscle in the solutions used always show themselves long before either tissue has permanently lost its irritability. Under all the conditions so far tried the smooth muscle remains alive longer than the striated; and the reactions of the former must therefore be regarded as, if anything, more 'normal' than those of the latter.

CHEMICAL EXPERIMENTS ON THE DIFFUSION OF SALT AND SUGAR THROUGH THE SURFACES OF THE SMOOTH MUSCLE FIBERS

The experiments on the changes of weight undergone by smooth muscle in various solutions should be supplemented by numerous others which will demonstrate directly by chemical analysis that salts and sugars diffuse through the surfaces of the living smooth muscle fibers; and by still others which will throw light on the sources of the potassium and phosphorus found in the smooth muscle ash. I hope to carry out such experiments in the not distant future. But such chemical experiments demand a great deal of time and material; it will, perhaps, be proper to give in this place some preliminary results from work in this field.

Experiments 58, 59, 62 and 63 give the results of an attempt to determine directly whether potassium, sodium, and cane sugar diffuse through the surfaces of the living smooth muscle fibers. Experiments 58 and 59 show that something more than 40 per cent of the tissue's sodium diffuses out into a surrounding sugar solution in five hours. The volume of such samples of tissue as were used in Experiments 58, 59, 62 and 63 consists to about 85 per cent of muscle fibers and to about 15 per cent of connective tissue and interstitial spaces. If the connective tissue and interstitial spaces be supposed to contain NaCl in the same concentration as the blood plasma,²⁷ the amount of the salt in these parts

²⁷ See Urano: *Zeitschrift für Biologie*, 1907, Bd. 50, pp. 218, 219, 224 and 225.

of the preparations would be 0.4×0.15 or 0.06 per cent of the weight of the whole fresh preparation. The total amount of NaCl in such preparations as those used in Experiments 58 and 59 is about 0.19 per cent. Forty per cent of this would be 0.19×0.4 , or 0.076 per cent of the weight of the whole fresh preparation. It would seem, therefore, as if more NaCl had been lost by the preparation than could be accounted for by supposing that all the NaCl contained in the connective tissue and interstitial spaces had diffused out into the sugar solution; and that some NaCl must, therefore, have been lost by the fibers.

I realize that the results of this computation ought not to be taken too seriously. The estimates of the relative volumes occupied by the fibers and other parts of the preparations used are rough, and the quantities of sodium dealt with in the analyses are small. On the other hand, it is very unlikely that all the NaCl would diffuse out from the connective tissue and interstitial spaces under the conditions of Experiment 59, and the experiment may, therefore, be regarded as *prima facie* evidence for the view that NaCl diffuses through the surfaces of the living smooth muscle fibers.

The case for the diffusion of cane sugar into the muscle fibers in Experiments 62 and 63 is much clearer. Experiment 62 shows that the samples of smooth muscle used contained 17.82 per cent of solid matter. • It may be supposed, therefore, that the tissue used in Experiment 63 contained 5.3976×0.1782 , or 0.9619 grams of solid matter. But its dry weight after about six hours stay in the 7.5 per cent sugar solution was 1.1422 grams, from which it would appear that $1.1422 - 0.9619$ or 0.1803 grams of sugar had diffused into the muscle.

The muscle treated with sugar solution contained at the end of its treatment $6.1854 - 1.1422$ or 5.0432 grams of water, into which had diffused 0.1803 grams or 3.6 per cent of sugar. That is to say, enough sugar had diffused into the muscle to bring the concentration of that substance in the muscle water up to 3.6 per cent; or, to look at the matter in another light, it may be said that a little less than half the water of the muscle was made up to a 7.5 per cent sugar solution. It is inconceivable that all this sugar

was contained in the connective tissue and interstitial spaces of the preparation.

It has been argued by Meigs and Ryan²⁸ from the sodium and chlorine content of smooth muscle that about half the water of the tissue is held by the colloids as organic water. It is very interesting to note that the amount of sugar which diffused into the muscle in Experiment 63 is less than, though not very far from, the amount which would be required to bring the concentration of that substance up to 7.5 per cent of the weight of the supposed quantity of inorganic water in the tissue.

Experiments 58 and 59 show that smooth muscle holds its potassium under even rather unfavorable conditions with remarkable tenacity. It is interesting to compare the results of these experiments with those of results obtained by Urano²⁹ and Fahr³⁰ in more or less similar experiments on striated muscle.

In my experiments no precautions whatever were taken in preparing the muscle, or to keep it near its normal state during its stay in sugar solution. The muscle was handled quite roughly in separating it from the mucous membrane, and still more roughly in freeing it from sub-mucous connective tissue. Each piece of muscle was cut along the line of the lesser curvature in order to open the stomach and then into a cardiac and pyloric portion. The room temperature during the preparation of the tissue was 22° and the temperature of the sugar solution in which it was kept varied between 22° and 25°. Finally the thin sheets of stomach muscle probably presented about the same amount of surface in relation to their volume as did the sartorii used by Urano and Fahr.

Urano found that about a third of the potassium of striated muscle would diffuse out into a sugar solution if the tissue were not carefully prepared. He accordingly used sartorii, which were carefully prepared and kept in cooled and oxygenated sugar solution; and, even under these conditions, the tissue lost about 24 per cent of its potassium in six hours. Fahr used in general

²⁸ Meigs and Ryan; *Jour. Biol. Chemistry*, 1912, xi, p. 491.

²⁹ Urano; *Zeitschrift für Biologie*, 1907, Bd. 50, p. 212; 1908, Bd. 51, p. 483.

³⁰ Fahr; *Zeitschrift für Biologie*, 1908, Bd. 52, p. 72.

the same technique as Urano, but prepared his sartorii still more carefully, and found that it lost about 6 per cent of its potassium in six hours. The smooth muscle used in my experiments and treated as above described lost only 4 per cent of its potassium in five hours.

These results at least indicate that the potassium of smooth muscle is held in the tissue in some way other than by such semi-permeable membranes as may be supposed to surround the striated muscle fibers.

THE CHANGES OF LENGTH UNDERGONE BY SMOOTH MUSCLE IN VARIOUS SOLUTIONS

In earlier articles the author has given evidence to show that the contraction of both striated and smooth muscle is the direct mechanical result of a change in the volume of certain histological components of the two tissues. It is probable that the contraction of striated muscle is caused by an increase in the volume of its sarcostyles; while that of smooth muscle is caused by a decrease in the volume of its fibers. These conclusions rest partly on the results of a microscopic examination of the two tissues in relaxation and contraction, partly on the fact that the two tissues may be made to change in length by immersing them in reagents which bring about changes in their volume.³¹

The relations between the changes in length and the changes in weight which occur in striated muscle as the result of immersing it in various solutions have already received a good deal of attention, and it has been shown that these relations are such as to indicate that any increase in the volume of the sarcostyles causes them to shorten.³²

In the experiments on the changes of weight undergone by smooth muscle in various solutions, which have been described in the preceding pages, the changes of length undergone by the muscle fibers have been roughly followed. It is usually easy to

³¹ See Meigs; *Zeitschrift für Allgemeine Physiologie*, 1908, Bd. 8, p. 81; *American Jour. Physiol.*, 1908, vol. 22, p. 477.

³² Meigs; *American Jour. Physiol.*, 1910, vol 26, p. 191.

do this by simple inspection of the piece of muscle, for the proportional change of length undergone by smooth muscle fibers is very considerable. I was careful, however, to make my judgments of the changes in length as objective as possible. Where the behavior of a single piece of tissue was being studied, the question whether the fibers had shortened or lengthened much or little was in each case decided before weighing. It was found that the amount of change in weight could in most cases, be predicted surprisingly accurately from a knowledge of the change which had taken place in the length of the muscle fibers. In many cases the changes undergone by two pieces of muscle, of which the fibers had about the same length at the start, were compared with each other. In a few cases, the length of the fibers of pieces of muscle was measured at various stages of the experiment.

It has been found to be a very general rule that increase in the weight of a piece of smooth muscle goes hand in hand with increase in the length of its fibers, while decrease in the weight of the muscle is accompanied by a decrease in the length of its fibers. For the evidence on this point, the reader is referred to the series of protocols of the experiments. Some of the cases are very striking. Pieces of smooth muscle, transferred from Ringer to half strength Ringer, for instance, gain weight and lengthen slowly; while pieces of smooth muscle, transferred from Ringer to double strength Ringer, lose weight and shorten rapidly (Experiments 33, 52, 55 and 57).

In isotonic NaCl and KCl solutions, smooth muscle undergoes a diphasic change of weight. In the NaCl solution it first gains weight and then loses, while in the KCl solution it first loses weight and then gains. In both cases increase in weight tends to be accompanied by lengthening of the muscle fibers and decrease in weight by shortening (Experiments 34, 35, 65 and 73). It will be noted that in these experiments the changes in weight and length do not run exactly parallel. In the NaCl solution the muscle begins to shorten a good while before it begins to lose weight, while in the KCl solution, it remains shortened for some time after it has begun to gain weight. These results are, however, just what should be expected on the supposition that an

increase in the volume of the individual fibers brings about an increase in their length and vice versa.

The NaCl and KCl solutions would not affect all the fibers of the preparation in the same way at the same time. In the NaCl solution, for instance, the outer fibers of the preparation would already have passed through the stage of swelling and lengthening, and would have begun to lose weight and shorten while the inner fibers were still swelling. And in the KCl solution, the outer fibers would begin to swell long before the inner ones had completed the stage of losing weight and shortening. As the preparations are allowed to lie in the solutions with their ends unattached, the shortening of a very few fibers would show itself as a shortening of the whole preparation, and this is no doubt the reason why the shortening begins in NaCl before the loss of weight is marked; and lasts far into the period of gain in weight in the KCl solution. In Experiment 35, the muscle was not examined until after it had been for more than three hours in the NaCl solution, and the period of marked lengthening is, therefore, not recorded.

It has already been said that smooth muscle varies in the amount of fluid which it takes up from Ringer's solution. Other things being equal, the tissue takes up more fluid at room temperature than at from 0° to 1° , and the muscle from the larger frogs of a given species tends to take up more fluid than that from the smaller frogs. In these cases also the changes in weight and length go hand in hand—the muscle from the larger frogs of a species lengthens more in Ringer's solution than that from the smaller frogs, and smooth muscle lengthens much more in Ringer's solution at room temperature than at from 0° to 1° (Experiments 18, 19, 20, 27, 31, 36 and 53).

Experiments 33, 55 and 57 show that when the mechanical stimulation which is inseparable from drying and weighing a piece of smooth muscle happens to cause it to shorten, it causes it also to lose weight. In the case of Experiment 52, the drying and weighing did not cause any noticeable shortening and failed also to cause the muscle to lose weight.

The changes of length undergone by smooth muscle in Ringer solution, in which small quantities of lactic acid have been sub-

stituted for the NaHCO_3 , are particularly interesting, because it is not improbable that lactic acid may play an important part in the physiology of the tissue. It has been shown on p. 525 that small quantities of lactic acid inhibit the tendency of smooth muscle to gain weight in Ringer's solution. The tendency for the fibers to lengthen is also inhibited by the acid (Experiments 27 and 28).

There are certain cases in which the rule that smooth muscle lengthens when it increases in weight and shortens when it decreases in weight does not hold. One of these has already been described in a previous article.³³ Pieces of smooth muscle immersed in slightly alkaline Ringer shorten without undergoing any marked decrease in weight. It has been shown, however, that under these circumstances the fibers lose fluid which is held by the muscle in the interstitial spaces. This case would not, therefore, be an exception to the rule that the shortening of smooth muscle fibers is accompanied by a decrease in their own volume.

Another partial exception is the case of muscle immersed in Ringer without NaHCO_3 to which moderately large amounts of lactic acid have been added—0.05 per cent or above. Pieces of frog's stomach muscle take up considerable quantities of fluid from such acid solutions without lengthening in proportion, though some lengthening often does occur (Experiment 24).

The action of such lactic acid solutions on smooth muscle is, however, rather complicated. Solutions which contain less than 0.025 per cent of acid create a tendency for the muscle fibers to lose weight and shorten. It is probable that when a piece of muscle is immersed in one of the stronger acid solutions, the acid reaches the inner fibers at first in a lower concentration and causes in them a tendency to shorten. Further, the acid produces a peculiar change in the consistency of the muscle, rendering it stiff and inextensible. It may well be that this setting of the muscle substance renders it incapable of undergoing any marked change in length.

³³ Meigs; *American Jour. Physiol.*, 1912, vol. 29, p. 317.

GENERAL DISCUSSION

The preceding pages of this article contain little more than an account of experiments, which, however, have an unmistakable bearing. They point to the view that the fibers of smooth muscle differ from those of striated muscle in not being surrounded by semi-permeable membranes; and they confirm the view, for which much independent evidence has already been adduced,³⁴ that the lengthening and shortening of smooth muscle fibers under physiological and other conditions result from increase and decrease in their volume, respectively.

Evidence that the smooth muscle fibers change in volume when immersed in various solutions

These conclusions cannot be drawn from the experiments, however, unless it can be shown that the changes of weight undergone by the smooth muscle preparations represent changes in the volume of the fibers. It is very desirable, therefore, to have as much evidence as possible on this point. It has already been shown that the smooth muscle fibers make up about 80 per cent of the volume of such preparations as were used in the experiments; the remainder consists roughly of equal parts of connective tissue and of spaces between the fibers which contain no formed histological elements.

These facts alone make it difficult to believe that the changes of weight undergone by the tissue in most of the experiments can be attributed to anything except changes in the volume of the fibers. The interstitial spaces and connective tissue would both have to double in volume in order to account for an increase of 20 per cent in the volume of the preparation as a whole, and that such a change would occur in say Ringer's solution is to say the least improbable. In other cases, as, for instance, where the preparation is immersed in double strength Ringer, it would have to be supposed that the interstitial spaces and connective tissue completely disappeared in order to account for the loss of weight which

³⁴ Meigs; American Jour. Physiol., 1908, vol. 22, p. 477; 1912, vol. 29, p. 317.

ensues. But the question is a very important one, and various experiments have been carried out with the view of answering it as completely as possible.

It will hardly be supposed that the changes in weight of the pieces of smooth muscle used in the experiments are to be ascribed to changes in the volume of the interstitial spaces, which contain no formed histological elements. Changes in the volume of these spaces would have to be very large in proportion to their original volume, in order to account for the most moderate change in weight of the whole preparation, and there is evidence to show that in Ringer's solution no such change in the volume of the interstitial spaces occurs. Cross sections of smooth muscle about 0.2 mm. thick have been kept for twelve hours or more in Ringer's solution and then examined microscopically. The interstitial spaces in such sections are not proportionally larger than in sections of smooth muscle from a freshly killed frog. The sections of muscle kept for twelve hours in Ringer's solution may sometimes be seen to contract when stimulated beneath the microscope.

Pieces of smooth muscle transferred from Ringer's solution to double strength Ringer often lose, as has been said, 20 per cent or more of their original weight. This loss could not be accounted for by a change in the volume of the interstitial spaces, even if it were supposed that these altogether disappeared. But if thin cross sections of muscle which have been for sometime in double strength Ringer be examined microscopically, it will be found that the interstitial spaces are relatively as large as or larger than the spaces in similar preparations of fresh muscle.

The experiments with connective tissue, described on pp. 526-528 make it difficult to believe that changes in the weight of this tissue play any considerable part in the changes of weight undergone by the smooth muscle preparations in various solutions.

The osmotic properties of striated muscle

My experiments with striated muscle confirm Overton's results with striated muscle except in the case of the action of isotonic KCl solutions. My results show that the weight of living striated muscle immersed in an isotonic KCl solution may increase 50 per cent; from which it follows that the surfaces of the living striated muscle fibers are quite permeable to KCl. The experiments with half strength and double strength Ringer solution, add to the already existing evidence for the view that the striated fibers are surrounded by semi-permeable membranes. They show that living muscle immersed in non-isotonic solutions gains or loses weight in such a manner as to suggest that osmosis plays the chief part in the early stages of the process.

While the evidence for the view that the surface of the striated muscle fiber is relatively impermeable to salts and sugars is very strong, there are equally strong reasons for believing that both the permeability of the surface and the osmotic pressure of the contents of the fiber are subject to variation. Some of the reasons for holding this view have already been given in the introduction, and my own experiments add still further to the evidence for it. The curves of figures 3, 4, 5 and 6 which show how striated muscle gains weight in half strength Ringer solution and loses weight in double strength Ringer solution, indicate that the osmotic pressure of the contents of the muscle fibers is subject to variation. It is to be noted that only the beginnings of these curves are of such a character as to suggest that the water intake or outflow is an osmotic process. After the first twenty or twenty-five minutes the curves representing these processes become nearly straight lines—the muscle absorbs or gives out equal quantities of fluid in equal periods of time.

It may readily be supposed that the initial osmotic intake of water from a hypotonic solution by the muscle and the consequent dilution of the salts within the fibers cause the muscle colloids to combine slowly with water. The water entering the muscle fibers would, therefore, combine with the colloids about as fast as it came in, the salts dissolved in the inorganic water would be maintained for some time at about the same concentration some-

what above that of the surrounding half strength Ringer solution, and during this period equal quantities of water would enter the muscle fibers in equal periods of time.

A generally similar explanation may be given for the curve which represents the loss of weight by the muscle in double strength Ringer solution. In this case the increasing concentration of the salts within the fibers due to the loss of inorganic water would cause the colloids to give up some of their organic water, and the curve of loss of weight would take the form of a straight line for the same reasons as in the case of the hypotonic solution.

It is interesting to observe that the curves representing the loss of weight by striated muscle in hypertonic solutions are more regular than those representing the gain of weight in hypotonic solutions (compare fig. 4 with figs. 5 and 6). The irritability of striated muscle is increased by immersion in hypotonic solutions and decreased by immersion in hypertonic solutions. After a few minutes' immersion in half strength Ringer the tissue often twitches as a result of the mechanical stimulation which accompanies the process of drying and weighing. It may well be that the irregular chemical activity which is expressed in this twitching is the cause of the irregularity of the later part of the curve of water intake.

Experiments 12, 17, 32 and 56 furnish evidence for the view that under certain conditions the muscle membranes may become somewhat permeable to the muscle salts, though the irritability of the tissue is not appreciably affected. It is shown in these experiments that striated muscle immersed in half strength Ringer first gains and then loses weight, though it remains quite irritable through the whole course of the experiment. The loss of weight may most readily be accounted for by supposing that some of the potassium phosphate, which gives to the muscle fluid its normal osmotic pressure, diffuses out of the fibers in the course of the experiment.

The situation with regard to striated muscle may be summed up by saying that osmosis plays an important part in the changes of weight which the tissue undergoes when immersed in various solutions, but that important parts are played by other factors also.

The osmotic properties of smooth muscle

Most of the arguments which indicate that the striated muscle fibers are surrounded by semi-permeable membranes fail in the case of smooth muscle. Smooth muscle always take up more fluid from Ringer's solution than the striated muscle from the same animal. It is difficult to show that smooth muscle has any more tendency to take up fluid from a half strength Ringer solution than from Ringer (compare Experiments 33 and 57 with Experiments 9, 19, 27 and 53). It is interesting to compare the behavior of the smooth muscle in Experiments 33 and 57 with that of the striated muscle from the same animals in Experiments 32 and 56 in both Ringer and half strength Ringer. In isotonic $\text{NaC}_2\text{H}_3\text{O}_2$ and K_2HPO_4 solutions smooth muscle behaves more or less as though its fibers were surrounded by semi-permeable membranes, but not at all so in isotonic NaCl and KCl solutions (Experiments 34, 35, 65, 67, 69 and 73). The curves which represent the changes of weight undergone by smooth muscle in hypotonic and hypertonic Ringer solution respectively, never in the least suggest that the taking up or loss of water could be an osmotic process (figs. 3 and 5). Smooth muscle takes up fluid rapidly from isotonic solutions of non-electrolytes, as though the surfaces of its fibers were highly permeable to those substances (Experiments 15, 46, 76 and 79). Finally, cutting across the fibers of smooth muscle seems to produce no change in the tissue, even in the immediate neighborhood of the cut (see pp. 506-507).

The swelling of smooth muscle in distilled water, in non-electrolytic solutions, and in isotonic and hypotonic salt solutions may most easily be explained by regarding it as an example of colloid swelling. The tissue swells in Ringer's solution more than in its normal medium. Under normal circumstances the tendency of the smooth muscle colloids to imbibe water is opposed by that of the colloids contained in the blood plasma and lymph; and this opposing tendency is absent from the Ringer solution. When smooth muscle is immersed in distilled water or in solutions of non-electrolytes, the salts dissolved in the fluids of its fibers diffuse out, and the loss of salt renders the muscle colloids more capable of swelling, as it does in the case of pieces of fibrin and gelatin

transferred from salt solutions to distilled water or to solutions of non-electrolytes. Smooth muscle loses fluid in hypertonic Ringer solution because the salts from this solution diffuse into the fibers and render the colloids less capable of imbibing or of holding water.

From Experiments 34, 35, 65, 67, 69 and 73 it appears that NaCl has a peculiarly strong effect in lessening the power of the smooth muscle colloids to absorb and hold water; that $\text{Na}_2\text{H}_2\text{O}_4$ has less effect in this direction; K_2HPO_4 , still less; and KCl, much less than any of the other three. Experiments 7, 33, 57 and 59 indicate that the smooth muscle colloids show very little increase in their tendency to absorb water until after they have lost a large proportion of their NaCl. In Experiment 59 the muscle had lost more than 40 per cent of its NaCl, yet had increased in weight only 0.07 per cent. In Experiments 33 and 57 small pieces of muscle which had already shown a marked tendency to gain weight in Ringer's solution, were only 18.1 per cent and 18.7 per cent heavier than originally after nineteen and twenty hours' immersion in half strength Ringer respectively. Experiments 9, 19, 27 and 53 show that pieces of smooth muscle often gain weight more than this in Ringer's solution. In Experiment 7, a small piece of muscle gained only 43.6 per cent in the course of twenty-three hours' immersion in a 0.2 per cent NaCl solution. This result is to be contrasted with those of Experiments 76 and 13 in which pieces of muscle gained 82 per cent and 144 per cent of their original weights in 3.95 per cent dextrose and distilled water respectively.

A consideration of Experiments 3, 13, 15, 46, 60, 61, 76 and 79 shows that non-electrolytes have an effect in inhibiting the tendency of the smooth muscle colloids to absorb water, particularly in the presence of small amounts of electrolytes. It is impossible to understand why the muscle of Experiment 61 should swell so little in the mixture of sugar solution with Ringer, unless the sugar, as well as the electrolytes of the mixture, has an effect in inhibiting the colloids from swelling. Experiments 3, 13, 15, 46, 76 and 79 show how much more rapidly and to how much greater extent smooth muscle swells in distilled water than in sugar solutions.

General physiology of striated and smooth muscle

The changes of length undergone by the smooth muscle fibers in the various solutions which have been experimented with, indicate that the structure of these fibers is such that any increase in their volume brings about an increase in their length. It must be pointed out that this conclusion and the others which have been reached concerning the osmotic properties of both striated and smooth muscle accord very well with certain earlier work on the histology and physiology of the two kinds of muscle.

The histological examination of striated muscle shows that it has a rather complicated structure. The smallest visible microscopic elements are the fibrillae or sarcostyles, which are bundled together to form the muscle fibers. The volume of these is made up to about equal parts of the sarcostyles and of the spaces between them, which are filled with a medium probably fluid called sarcoplasm. The muscle fiber is surrounded by a histologically demonstrable membrane, the sarcolemma. Between the muscle fibers are spaces filled with lymph.

Histological examination of vertebrate smooth muscle has shown that its so-called fibers cannot be regarded as bundles of smaller elements comparable to the striated sarcostyles. The fresh fibers appear homogeneous³⁵ and the fixed fibers show little more sign of inner structure than does any piece of coagulated protoplasm.³⁶

It is now well established that in fixed contracted specimens of striated muscle the sarcostyles are relatively larger and the sarcoplasmic spaces smaller than in uncontracted specimens; while in fixed contracted preparations of smooth muscle the fibers are relatively smaller and the interstitial spaces relatively larger.³⁷ The author has advocated the view that these facts mean that fluid passes from the sarcoplasmic spaces to the sarcostyles in

³⁵ Engelmann; *Archiv für die gesammte Physiologie*, 1881, Bd. 25, p. 546.

³⁶ Meigs; *American Jour. Physiol.*, 1908, vol. 22, pp. 482, et seq.

³⁷ See Hürthle; *Biologisches Centralblatt*, 1907, Bd. 27, pp. 122-124; Meigs, *Zeitschrift für allgemeine Physiologie*, 1908, Bd. 8, p. 81; *American Jour. Physiol.*, 1908, vol. 22, p. 477; Guthertz, *Archiv für mikroskopische Anatomie und Entwicklungsgeschichte*, 1910, Bd. 75, p. 209; Heiderich; *Anatomische Hefte*, 1902, Bd. 19, p. 451; Eycleshymer, *Am. Jour. Anat.*, 1904, vol. 3, p. 293.

striated muscle during contraction, and from the fibers to the interstitial spaces during the contraction of smooth muscle. The microscopic examination of fresh smooth muscle points to the same conclusion.³⁸

It would appear, then, that during the contraction of smooth muscle there is an exchange of fluid between the cells of the tissue and their surroundings. In the case of striated muscle also a transfer of fluid occurs during contraction, but this is entirely intra-cellular. Semi-permeable membranes surrounding the striated muscle fibers would not interfere with the intra-cellular exchange of fluid between the sarcoplasmic spaces and the sarcostyles. But it would be difficult to understand how the smooth muscle fibers could lose fluid during every contraction and take it up again during every relaxation if they were surrounded by membranes which were impermeable to dissolved salts.

Striated muscle swells and shortens in distilled water, and the shortening and swelling may be removed together by transferring the muscle to 0.7 per cent NaCl solution.³⁹ But the muscle may be made to swell without shortening or to shorten without swelling.⁴⁰ A careful consideration of the experiments which have been carried out along these lines shows that it is not possible to make the striated muscle go into a condition of marked permanent shortening without seriously injuring it or killing it; only in dead muscle is there a close relation between increase in weight and decrease in length. These facts receive a ready explanation from the view that the shortening of striated muscle is caused by the swelling of its sarcostyles which are enclosed by the semi-permeable membranes of the fibers, but not individually surrounded by such membranes. It is easy to see how there might be in living muscle under many conditions, a tendency toward a change in the volume of the sarcoplasmic spaces without any corresponding tendency toward a change in the volume of the sarcostyles. In dead muscle, on the other hand, the semi-permeable membranes are destroyed, the sarcoplasmic fluid escapes, and the sarcostyles come into comparatively close rela-

³⁸ Meigs; *American Jour. Physiol.*, 1912, vol. 29, p. 317.

³⁹ Meigs; *American Jour. Physiol.*, 1910, vol. 26, p. 191.

⁴⁰ Meigs; *English Jour. Physiol.*, 1909, vol. 39, p. 385.

tions with surrounding solutions. Under these conditions a change in the weight of the tissue as a whole would be much more likely to mean a change in the volume of the sarcostyles than under the conditions which exist while the tissue is still alive.

It has been shown on pp. 532-535 of this article that changes of length and volume run much more closely parallel in living smooth muscle than in living striated muscle. This is easy to understand if it may be supposed that changes in the length of the smooth muscle fibers depend on exchanges of fluid between them and their surroundings.

Many aspects of the experiments which have been described in this article acquire a new meaning in the light of these considerations. Striated muscle immersed in distilled water first gains in weight, then loses, then gains again, and finally loses again (fig. 16).⁴¹ This peculiar behavior is readily explained by supposing that the first gain is the expression of an osmotic intake of water which results in the production of lactic acid and the destruction of the semi-permeable membranes. The lactic acid causes the sarcostyles to swell slowly, but this swelling is more than offset in the second period of the curve by the loss of fluid from the sarcoplasmic spaces. The second period of gain in weight is the expression of the slow continued swelling of the sarcostyles after the sarcoplasmic fluid has escaped; and the second period of loss, of a slow loss of fluid by the sarcostyles due to the gradual escape of lactic acid to the surrounding solution. Smooth muscle immersed in distilled water simply gains and then slowly loses weight; it reacts as would the sarcostyles of striated muscle if they were deprived of their surrounding sarcoplasm and semi-permeable membranes (fig. 16). Attention may also be called to the character of the curves which represent the swelling of smooth muscle in distilled water and hypotonic solutions, and to the manner in which the smooth muscle takes up fluid from solutions of non-electrolytes. In all these particulars it resembles dead striated muscle much more closely than living striated muscle (figs. 3, 4, 5, 6, 9, 10, 11, 12, 14, 15, 16 and 20).

It seems at first sight strange that the chemical processes which bring about contraction should do so in striated muscle by causing

⁴¹ See also Fischer, *Archiv für die gesammte Physiologie*, 1908, Bd. 124, p. 74.

the sarcostyles to swell; and in smooth muscle, by causing the fibers to lose fluid. Experiments 23, 28, 39 and 44 show how the production of lactic acid might bring about both sets of results.

It has long been known that striated muscle swells in weak acid solutions, and this fact has been vaguely taken to mean that tissues generally swell in such solutions. It is now well known, however, that striated muscle may produce large amounts of lactic acid on its own account when placed under abnormal conditions, and it is impossible to distinguish the swelling produced by the external acid from that caused by the muscle's own yield.

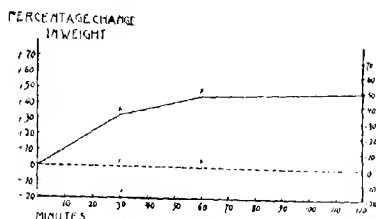


Fig. 20 Changes in weight undergone by a living sartorius (broken line) and by a dead sartorius (unbroken line) in 7.5 per cent cane sugar solution. See Experiments 5 and 6.

The experiments with connective tissue furnish the possibility of determining how strong an acid solution must be before it can produce swelling in this tissue, and they show that the same concentration of acid which promotes swelling in the connective tissue inhibits it in the case of the smooth muscle.

It may be, then, that stimulation causes smooth muscle to produce very small quantities of lactic acid, and that the acid production results in a tendency for the muscle fibers to lose fluid and shorten.

This would seem to contradict the general rule which has been set up by the work of Wolfgang Ostwald, Lillie, Fischer and others⁴² to the effect that colloids tend to swell more in acids and

⁴² See Ostwald; *Archiv für die gesamte Physiologie*, 1905, Bd. 108, p. 563, Bd. 109, p. 277; Lillie, *American Jour. Physiol.*, 1907, vol. 20, p. 127; Fischer, *Archiv für die gesamte Physiologie*, 1908, Bd. 124, p. 69; Bd. 125, p. 99.

alkalies than in neutral solutions. Ostwald has found, however, that very small quantities of acid inhibit the tendency of gelatin to absorb water, while larger quantities increase this tendency. In the case of smooth muscle, it may be supposed that the reaction of the tissue is normally slightly alkaline, and that the production of such quantities of acid as can be formed under physiological conditions tends only to bring their reaction toward the neutral point. This view is to some extent confirmed by the fact that quantities of acid above 0.025 per cent cause pieces of smooth muscle to take up fluid from Ringer's solution (Experiments 23 and 24).

Such results as those of Experiments 33, 55 and 57, in which it is shown that mechanical stimulation produces a tendency for smooth muscle to shorten and lose weight, receive a ready explanation if it be supposed that the stimulation causes the production of small amounts of lactic acid. And the same explanation may be applied to the results of Experiments 9, 27 and 36 in which it is shown that the tissue acquires a tendency to lose weight and shorten after it has been immersed for a considerable period in Ringer's solution. Still further evidence pointing in the same direction is to be obtained from Experiments 16 and 23, in which it is shown that after pieces of smooth muscle have been for some time in a weak acid solution or in Ringer without NaHCO_3 , stimulation causes them to lengthen, or at least that lengthening is the much more obvious effect of stimulation. It is a very tempting hypothesis that after the reaction of the muscle fibers has been rendered acid the production of still further acid as the result of stimulation causes them to take up fluid and lengthen.

The results of Experiments 19 and 20 in which it is shown that smooth muscle takes up more fluid from Ringer's solution at higher temperatures, are more or less opposed to these theoretical views. It is difficult to suppose that smooth muscle produces less lactic acid at higher temperatures than at lower ones. But it must be remembered that connective tissue also takes up fluid from Ringer's solution more rapidly at higher temperatures (Experiments 47 and 48) and this indicates that the greater tend-

ency to swell at higher temperatures may be a peculiarity of certain colloids independent of any physiological activity. It may be supposed, therefore, that the colloids of frog's smooth muscle tend to swell at higher temperatures and that this tendency is, at any rate at first, sufficient to overcome any tendency toward loss of fluid which may be produced by more rapid lactic acid formation at the higher temperature.

The fact that the smooth muscle lengthens at the same time that it swells in the Ringer solution at the higher temperature shows that high temperatures must produce in the tissue some change other than that which follows stimulation and results in shortening. And it is interesting to remember in this connection that the tendency to lengthen at temperatures above 40° is not a general characteristic of smooth muscle. Vincent and Lewis have shown that in mammalian smooth muscle heating produces shortening somewhat as it does in striated muscle.⁴ It would be difficult to believe that heating produced chemical changes of an opposite character in frog's muscle and in mammalian muscle; but, if it may be supposed that heating produces in both tissues a tendency for the colloids to swell and at the same time a tendency toward lactic acid production, it would be easy to explain the opposite reactions of the two tissues by supposing that in frog's muscle the former change preponderated; and in mammalian muscle, the latter.

GENERAL CONCLUSIONS

This article may be concluded by the statement of certain views regarding the physiology of muscle and of muscular contraction which are indicated or confirmed by the experimental results which have been presented.

Striated muscle consists of fibers surrounded by semi-permeable membranes. The fibers are made up of sarcostyles and sarcoplasm; the sarcostyles are fibrils with a characteristic structure which run longitudinally through the fiber and are separated from each other by the fluid sarcoplasm. The sarcostyles are not separated from the sarcoplasm by semi-permeable membranes;

⁴ Vincent and Lewis; *Jour. Physiol.*, 1901, vol. 26, p. 445.

the physical relations between sarcostyles and sarcoplasm are in general similar to those which obtain between a piece of gelatin and a surrounding watery solution in which it is immersed.

The striated muscle fibers are separated from each other by spaces filled with lymph. The osmotic pressure of the salts of the lymph is opposed within the muscle fibers by that of diffusible potassium phosphate in solution in the sarcoplasm. The muscle fibers contain a certain amount of water which is combined with colloids and cannot act as a solvent for salts. A large proportion, if not all, of this organic water is probably contained in the sarcostyles.

Stimulation of the muscle causes it to produce lactic acid. The presence of the acid brings about a tendency for the sarcostyles to swell at the expense of the sarcoplasmic spaces, and the shortening of the muscle fiber is the direct mechanical result of the increase in the volume of its sarcostyles. Relaxation of the muscle may be brought about by the combination of the lactic acid with the K_2HPO_4 contained in the muscle fibers, and its consequent neutralization.

Smooth muscle consists of fibers which have an internal homogeneous structure and are not surrounded by semi-permeable membranes. The smooth muscle fibers are separated from each other by spaces containing lymph, and the physical relations between the lymph and the smooth muscle fibers are generally similar to those which obtain between the sarcostyles and the sarcoplasm.

The smooth muscle fibers contain a larger proportion of organic water than the striated ones, and the remaining inorganic water contains NaCl in the same concentration as that in which it exists in the lymph. The tendency of the fibers to swell or to lose fluid is dependent among other things on the concentration of NaCl contained in the inorganic water.

Stimulation of the smooth muscle causes it to produce lactic acid, but in very much smaller quantity than is produced under similar circumstances by striated muscle. The presence of small quantities of the acid in smooth muscle brings about a tendency for its fibers to give up fluid to the intervening lymph spaces, and

the shortening of the smooth muscle fibers is the direct mechanical result of this decrease in volume.

The question how the relaxation of smooth muscle is brought about is bound up with the question how the presence of acid could decrease the tendency of the smooth muscle colloids to take up or hold water; and on this question there is as yet practically no experimental evidence. But the supposition that the acid acts to neutralize an already present slight alkalinity of the colloids suggests an interesting explanation of certain of the physiological peculiarities of smooth muscle. It may be supposed that the combination of the acid with the alkaline colloid is comparatively stable, and that the resulting state of decreased alkalinity can be made to disappear only by some further slow chemical process by which the lactic acid radicle is either further oxidized or otherwise got rid of. This supposition would furnish a preliminary rough explanation of the slowness with which relaxation often occurs in smooth muscle, and of the tissue's power of remaining for an indefinite period in a state of high tonic contraction.

PROTOCOLS OF THE EXPERIMENTS

EXPERIMENT 1

November 3, 1909

Sartorius from frog 1, weighed fresh 0.082 gram

11.53 A.M. immersed in distilled water	3.53 P.M. weighed 0.127 gram
12.23 P.M. weighed 0.135 gram	6.10 P.M. weighed 0.129 gram
12.53 P.M. weighed 0.121 gram	7.59 P.M. weighed 0.128 gram
1.53 P.M. weighed 0.120 gram	9.14 P.M. weighed 0.125 gram
2.53 P.M. weighed 0.126 gram	10.38 A.M. Nov. 4, weighed 0.124 gram

Temperature varied between 19° and 20°

EXPERIMENT 2

November 29, 1909

Sartorius from frog 2, weighed fresh 0.151 gram

10.55 A.M. immersed in distilled water	11.20 A.M. weighed 0.246 gram
11.00 A.M. weighed 0.218 gram	11.25 A.M. weighed 0.238 gram
11.05 A.M. weighed 0.238 gram	11.40 A.M. weighed 0.226 gram
11.10 A.M. weighed 0.248 gram	11.55 A.M. weighed 0.215 gram
11.15 A.M. weighed 0.251 gram	

Temperature varied between 19° and 20°

EXPERIMENT 3

December 6, 1909

Half of muscle from stomach of frog 3, weighed fresh 0.086 gram
 2.05 P.M. immersed in distilled water 2.35 P.M. weighed 0.204 gram
 2.10 P.M. weighed 0.102 gram 2.50 P.M. weighed 0.225 gram
 2.15 P.M. weighed 0.120 gram 3.05 P.M. weighed 0.231 gram
 2.20 P.M. weighed 0.131 gram 3.20 P.M. weighed 0.226 gram
 2.25 P.M. weighed 0.155 gram 9.52 A.M. Dec. 7, weighed 0.185 gram
 2.30 P.M. weighed 0.179 gram

Temperature varied between 19° and 21°

EXPERIMENT 4

December 8, 1909

10.34 A.M. Sartorius from frog 4, immersed in distilled water
 10.49 A.M. still slightly irritable
 10.59 A.M. apparently entirely unirritable; transferred to Ringer
 11.59 A.M. still entirely unirritable except in a very small portion at thick upper end
 3.59 P.M. still entirely unirritable except in a very small portion at thick upper end
 10.25 A.M. December 9, still entirely unirritable except in a very small portion at thick upper end
 12.01 P.M. weighed 0.190 gram 12.33 P.M. weighed 0.271 gram
 12.03 P.M. transferred to distilled water 12.38 P.M. weighed 0.285 gram
 12.08 P.M. weighed 0.198 gram 12.43 P.M. weighed 0.286 gram
 12.13 P.M. weighed 0.210 gram 12.48 P.M. weighed 0.290 gram
 12.18 P.M. weighed 0.226 gram 12.53 P.M. weighed 0.298 gram
 12.23 P.M. weighed 0.243 gram 12.58 P.M. weighed 0.295 gram
 12.28 P.M. weighed 0.256 gram 1.53 P.M. weighed 0.304 gram

Temperature varied between 15° and 19°

EXPERIMENT 5

January 15, 1910

Sartorius from frog 5, weighed fresh 0.122 gram.
 11.53 A.M. immersed in 7.5 percent cane sugar solution
 12.23 P.M. weighed 0.122 gram
 12.53 P.M. weighed 0.122 gram
 Temperature varied between 20° and 21°

EXPERIMENT 6

January 15, 1910

11.27 A.M. Sartorius from frog 5, immersed in distilled water
 12.27 P.M. transferred to Ringer's solution
 2.36 P.M. weighed 0.140 gram
 2.38 P.M. transferred to 7.5 per cent cane sugar solution
 3.08 P.M. weighed 0.187 gram
 3.38 P.M. weighed 0.204 gram
 4.38 P.M. weighed 0.216 gram
 Temperature varied between 20° and 21°

EXPERIMENT 7

October 14, 1910

Muscle from stomach of frog 6, weighed fresh 0.156 gram
 12.00 M. immersed in 0.2 per cent NaCl solution
 12.10 P.M. weighed 0.178 gram
 12.20 P.M. weighed 0.191 gram
 12.30 P.M. weighed 0.202 gram
 12.40 P.M. weighed 0.212 gram
 12.50 P.M. weighed 0.217 gram
 1.00 P.M. weighed 0.219 gram, fibers very much lengthened
 4.20 P.M. weighed 0.228 gram, fibers still very much lengthened
 11.20 A.M. October 15, weighed 0.224 gram, fibers still very much lengthened
 The temperature in this experiment was, unfortunately, not recorded, but was probably not far from 20°

EXPERIMENT 8

December 10, 1910

Sartorius from leopard frog 7, weighed fresh 0.088 gram
 12.28 P.M. immersed in Ringer
 6.03 P.M. weighed 0.085 gram
 11.17 A.M. December 12, weighed 0.089 gram
 Rigor just beginning, but muscle is still quite irritable
 Temperature varied between 16° and 20°

EXPERIMENT 9

December 10, 1910

Stomach muscle from leopard frog 7, weighed fresh 0.110 gram
 10.33 A.M. immersed in Ringer
 11.43 A.M. weighed 0.120 gram, fibers lengthened
 12.30 P.M. weighed 0.125 gram
 6.09 P.M. weighed 0.143 gram
 11.12 A.M. December 12, weighed 0.131 gram, fibers still lengthened; muscle still quite irritable
 Temperature varied between 16° and 20°

EXPERIMENT 10

December 13, 1910

Stomach muscle from leopard frog 8,¹ weighed fresh 0.056 gram
 10.19 A.M. immersed in Ringer
 11.19 A.M. weighed 0.060 gram, fibers markedly lengthened
 12.48 P.M. weighed 0.061 gram
 4.14 P.M. weighed 0.060 gram, fibers still lengthened
 10.12 A.M. December 14, weighed 0.061 gram, fibers very much lengthened and still highly irritable
 Temperature varied between 17° and 19°

¹ This frog weighed 14.2 grams.

EXPERIMENT 11

December 13, 1910

Stomach muscle from leopard frog 9,² weighed fresh 0.065 gram
 10.31 A.M. immersed in Ringer without NaHCO₃
 11.31 A.M. weighed 0.072 gram, fibers considerably lengthened
 12.58 P.M. weighed 0.074 gram
 4.18 P.M. weighed 0.082 gram, fibers markedly lengthened
 10.17 A.M. December 14, weighed 0.085 gram, fibers enormously lengthened and still highly irritable
 Temperature varied between 17° and 19°

² This frog weighed 12.7 grams.

EXPERIMENT 12

December 13, 1910

Sartorius of green frog 10, weighed fresh 0.148 gram

11.02 A.M. immersed in Ringer	12.42 P.M. weighed 0.168 gram
12.02 P.M. weighed 0.144 gram, transferred to half strength Ringer	12.52 P.M. weighed 0.171 gram
12.12 P.M. weighed 0.152 gram	4.08 P.M. weighed 0.182 gram
12.22 P.M. weighed 0.159 gram	10.07 A.M. December 14, weighed 0.172 gram, still highly irritable
12.32 P.M. weighed 0.164 gram	

Temperature varied between 17° and 19°

EXPERIMENT 13

December 15, 1910

Stomach muscle of green frog 11, weighed fresh 0.107 gram

10.11 A.M. immersed in distilled water	11.07 A.M. weighed 0.252 gram
10.19 A.M. weighed 0.130 gram	11.22 A.M. weighed 0.263 gram
10.27 A.M. weighed 0.149 gram	11.37 A.M. weighed 0.261 gram
10.35 A.M. weighed 0.172 gram	2.23 P.M. weighed 0.252 gram
10.43 A.M. weighed 0.188 gram	4.12 P.M. weighed 0.253 gram
10.51 A.M. weighed 0.217 gram, fibers enormously lengthened	9.59 A.M. December 16, weighed 0.240 gram
10.59 A.M. weighed 0.240 gram	

Temperature varied between 18° and 21°

EXPERIMENT 14

December 16, 1910

Sartorius of green frog 12, weighed fresh 0.102 gram

11.12 A.M. immersed in 7.5 per cent cane sugar solution
11.20 A.M. weighed 0.100 gram
11.28 A.M. weighed 0.102 gram
11.36 A.M. weighed 0.103 gram
11.44 A.M. weighed 0.105 gram
11.52 A.M. weighed 0.105 gram
12.00 M. weighed 0.104 gram
1.30 P.M. weighed 0.105 gram
2.30 P.M. weighed 0.104 gram
4.25 P.M. weighed 0.104 gram
9.45 A.M. December 17, weighed 0.105 gram, fibers somewhat shortened and entirely unirritable

10.00 A.M. Muscle transferred to Ringer
10.20 A.M. Still unirritable
11.20 A.M. Still unirritable

Temperature varied between 16° and 19°

EXPERIMENT 15

December 16, 1910

Stomach muscle of green frog 12, weighed fresh 0.133 gram

11.16 A.M. immersed in 7.5 per cent cane sugar solution
11.24 A.M. weighed 0.143 gram
11.32 A.M. weighed 0.155 gram
11.40 A.M. weighed 0.158 gram
11.48 A.M. weighed 0.169 gram
11.56 A.M. weighed 0.167 gram
12.04 P.M. weighed 0.173 gram
1.34 P.M. weighed 0.214 gram
2.34 P.M. weighed 0.215 gram
4.29 P.M. weighed 0.223 gram
9.49 A.M. December 17, weighed 0.227 gram, fibers much lengthened; muscle entirely un-irritable

10.17 A.M. transferred to Ringer
10.48 A.M. muscle now somewhat irritable; replaced in Ringer
11.15 A.M. muscle now highly irritable

Temperature varied between 16° and 19°

EXPERIMENT 16

December 19, 1910

Stomach muscle of leopard frog 13, weighed fresh 0.152 gram
 12.00 M. Immersed in Ringer without NaHCO_3
 12.20 P.M. weighed 0.161 gram, fibers somewhat lengthened
 3.10 P.M. weighed 0.173 gram
 5.25 P.M. weighed 0.171 gram
 10.20 A.M. December 20, weighed 0.168 gram, fibers much lengthened and still quite irritable
 9.40 A.M. December 21, fibers showed tendency to lengthen on stimulation¹
 Temperature varied between 17° and 21°

¹ See p. 509.

EXPERIMENT 17

December 20, 1910

Sartorius of green frog 14, weighed fresh 0.124 gram
 10.08 A.M. immersed in Ringer
 11.14 A.M. weighed 0.117 gram
 11.22 A.M. weighed 0.115 gram
 11.30 A.M. weighed 0.115 gram, transferred to half strength Ringer
 11.38 A.M. weighed 0.122 gram
 11.46 A.M. weighed 0.127 gram
 11.54 A.M. weighed 0.130 gram
 12.02 P.M. weighed 0.134 gram
 12.32 P.M. weighed 0.139 gram
 2.14 P.M. weighed 0.147 gram
 4.14 P.M. weighed 0.147 gram
 10.14 A.M. December 21, weighed 0.136 gram, still highly irritable
 Temperature varied between 18° and 20°

EXPERIMENT 18

January 10, 1911

Stomach muscle of leopard frog 15, weighed fresh 0.160 gram
 10.27 A.M. immersed in Ringer
 11.27 A.M. weighed 0.179 gram, fibers somewhat lengthened
 12.27 P.M. weighed 0.187 gram
 2.33 P.M. weighed 0.202 gram, fibers much lengthened
 4.33 P.M. weighed 0.202 gram
 11.33 A.M. January 11, weighed 0.210 gram, fibers still lengthened and highly irritable; temperature varied between 19° and 23°

EXPERIMENT 19

January 11, 1911

Portion of stomach muscle of leopard frog 16, weighed fresh 0.063 gram
 11.15 A.M. immersed in Ringer at room temperature (see below)
 12.15 P.M. weighed 0.072 gram, fibers slightly lengthened
 3.15 P.M. weighed 0.076 gram, fibers considerably lengthened
 5.15 P.M. weighed 0.077 gram, fibers still much lengthened
 9.55 A.M. January 12, weighed 0.077 gram, fibers still much lengthened; muscle still highly irritable
 Temperature varied between 20° and 21°

EXPERIMENT 21

January 13, 1911

Sartorius of green frog 17, weighed fresh 0.195 gram
 10.45 A.M. immersed in 4.34 per cent dextrose; went immediately into maintained contraction
 11.15 A.M. weighed 0.188 gram, contraction now less marked
 11.45 A.M. weighed 0.184 gram
 2.45 P.M. weighed 0.166 gram, fibers somewhat shorter than at 11.15 and entirely unirritable; muscle transferred to Ringer
 3.10 P.M. muscle now quite irritable
 4.00 P.M. muscle somewhat more irritable than at 3.10
 Temperature varied between 20° and 21°

EXPERIMENT 20

January 11, 1911

Portion of stomach muscle of leopard frog 16, weighed fresh 0.074 gram
 11.20 A.M. immersed in Ringer at 1°
 12.20 P.M. weighed 0.079 gram, fibers somewhat shortened
 3.20 P.M. weighed 0.081 gram, fibers still shortened
 5.20 P.M. weighed 0.082 gram, fibers still shortened
 Temperature of Ringer up to this point varied between 0° and 1°
 10.00 A.M. January 12, weighed 0.088 gram, fibers slightly longer than at last weighing, and still highly irritable
 From 5.20 P.M., January 11, to 10.00 A.M., January 12, temperature of Ringer varied between 0° and 8°

EXPERIMENT 22

January 13, 1911

Portion of stomach muscle of green frog 17, weighed fresh 0.248 gram
 10.55 A.M. immersed in 4.34 per cent dextrose
 11.10 A.M. fibers shortened
 11.25 A.M. weighed 0.277 gram, fibers have become slightly longer
 11.55 A.M. weighed 0.294 gram, fibers now decidedly lengthened
 2.55 P.M. weighed 0.288 gram, fibers still lengthened
 3.15 P.M. muscle still fairly irritable
 Temperature in preceding part of experiment varied between 20° and 21°
 At 3.55 P.M. a piece of this stomach muscle was cut off and weighed 0.139 gram. It was left in the dextrose solution at between 20° and 22° until 10.35 A.M. January 14, when it weighed 0.116 gram. Its fibers were rather shorter than at last weighing and still slightly irritable. Transfer to Ringer did not increase their irritability.

EXPERIMENT 23

January 19, 1911

Cardiac half of stomach muscle of leopard frog 18, weighed fresh 0.044 gram

2.05 P.M. immersed in Ringer without NaHCO_3 + 0.025 per cent lactic acid

2.35 P.M. weighed 0.044 gram, fibers somewhat lengthened

3.05 P.M. weighed 0.042 gram, still highly irritable

4.05 P.M. weighed 0.042 gram, only very slightly irritable

10.05 A.M. January 20, weighed 0.047 gram, shows small but decided tendency to lengthen on stimulation,¹ fibers not much changed in length since 2.35 P.M. yesterday

Temperature varied between 18° and 21°

¹ See p. 509.

EXPERIMENT 24

January 19, 1911

Pyloric half of stomach muscle of leopard frog 18, weighed fresh 0.055 gram

2.00 P.M. immersed in Ringer without NaHCO_3 + 0.05 per cent lactic acid

2.30 P.M. weighed 0.059 gram, fibers somewhat lengthened and still highly irritable

3.00 P.M. weighed 0.059 gram, still highly irritable

4.00 P.M. weighed 0.060 gram, no longer irritable; shows no tendency to lengthen on stimulation

10.00 A.M. January 20, weighed 0.067 gram, fibers still somewhat lengthened (about 30 per cent longer than those of cardiac half), and entirely unirritable

Temperature varied between 18° and 21°

EXPERIMENT 25

January 23, 1911

Piece of stomach muscle of bull-frog 19, weighed fresh 0.375 gram

5.00 P.M. immersed in 7.5 per cent cane sugar solution at 7°

5.30 P.M. weighed 0.423 gram

10.30 A.M. January 24, weighed 0.467 gram, fibers somewhat lengthened

11.30 A.M. weighed 0.518 gram

4.30 P.M. weighed 0.622 gram, fibers now much lengthened

10.30 A.M. January 26, weighed 0.598 gram, fibers still much lengthened and still quite irritable

11.00 A.M. January 27, muscle still barely irritable

11.10 A.M. immersed in Ringer

5.10 P.M. muscle now no longer irritable

Temperature during this whole experiment varied between 5° and 9°

EXPERIMENT 26

January 26, 1911

Sartorius of large bull-frog 20,¹ weighed fresh 0.602 gram

5.20 P.M. immersed in Ringer

6.20 P.M. weighed 0.694 gram

10.20 A.M. January 27, weighed 0.704 gram, still highly irritable

10.25 A.M. January 28, weighed 0.725 gram. Rigor is fairly well started though fibers are still somewhat irritable, particularly toward knee-end

Temperature varied between 19° and 22°

¹ This frog weighed over 150 grams

EXPERIMENT 27

January 26, 1911

Strip of stomach muscle from large bull-frog 20,¹ weighed fresh 0.120 gram
 4.30 P.M. immersed in Ringer
 5.30 P.M. weighed 0.131 gram, fibers somewhat lengthened
 9.30 A.M. January 27, weighed 0.143 gram, fibers somewhat longer than at last weighing; still highly irritable
 10.15 A.M. January 28, weighed 0.133 gram, fibers shortened and still fairly irritable

Temperature varied between 20° and 22°

¹ This frog weighed over 150 grams.

EXPERIMENT 28

January 26, 1911

Strip of stomach muscle from large bull-frog 20,² weighed fresh 0.113 gram
 4.35 P.M. immersed in Ringer without NaHCO₃, to which had been added 0.01 per cent lactic acid
 5.35 P.M. weighed 0.117 gram, fibers somewhat lengthened
 9.35 A.M. January 27, weighed 0.112 gram, fibers shorter than at last weighing
 10.55 A.M. still somewhat irritable
 10.20 A.M. January 28, weighed 0.103 gram, fibers still shortened and no longer irritable

Temperature varied between 20° and 22°

² This frog weighed over 150 grams.

EXPERIMENT 29

January 30, 1911

Tendo Achillis of leopard frog 21, weighed fresh 0.025 gram
 11.05 A.M. immersed in 7.5 per cent cane sugar solution
 11.50 A.M. weighed 0.029 gram
 2.07 P.M. weighed 0.029 gram
 4.07 P.M. weighed 0.029 gram
 10.07 A.M. January 31, weighed 0.028 gram
 Temperature varied between 19° and 22°

EXPERIMENT 30

January 31, 1911

Sartorius of small bull-frog 22,¹ weighed fresh 0.161 gram
 12.55 P.M. immersed in Ringer
 1.25 P.M. weighed 0.154 gram
 3.55 P.M. weighed 0.152 gram
 11.55 A.M. February 1, weighed 0.145 gram, still highly irritable
 Temperature varied between 19° and 21°

¹ This frog weighed 40.3 grams.

EXPERIMENT 31

January 31, 1911

Stomach muscle of small bull-frog 22,² weighed fresh 0.331 gram
 12.15 P.M. immersed in Ringer
 12.45 P.M. weighed 0.320 gram, fibers not lengthened
 3.15 P.M. weighed 0.327 gram, fibers slightly lengthened
 11.15 A.M. February 1, weighed 0.355 gram, fibers somewhat longer than at last weighing, and still very highly irritable

Temperature varied between 19° and 21°

² This frog weighed 40.3 grams.

EXPERIMENT 32

February 8, 1911

Sartorius of bull-frog 23, weighed
fresh 0.152 gram
11.52 A.M. immersed in Ringer
1.30 P.M. weighed 0.143 gram
2.00 P.M. weighed 0.142 gram
2.08 P.M. weighed 0.140 gram
2.16 P.M. weighed 0.1405 gram
2.24 P.M. weighed 0.140 gram
2.32 P.M. weighed 0.140 gram
2.40 P.M. weighed 0.141 gram
2.48 P.M. weighed 0.139 gram, trans-
ferred to half strength
Ringer
2.56 P.M. weighed 0.148 gram
3.04 P.M. weighed 0.153 gram
3.12 P.M. weighed 0.1565 gram
3.20 P.M. weighed 0.159 gram
3.28 P.M. weighed 0.1615 gram, still
very highly irritable
9.38 A.M. February 9, weighed 0.178
gram, still highly irritable
1.38 P.M. weighed 0.174 gram, still
very highly irritable
Temperature varied between 18° and
19°

EXPERIMENT 33

February 8, 1911

Stomach muscle of bull-frog 23,
weighed fresh 0.260 gram
12.00 P.M. immersed in Ringer
1.34 P.M. weighed 0.271 gram, fibers
considerably lengthened
2.04 P.M. weighed 0.260 gram
2.12 P.M. weighed 0.252 gram
2.20 P.M. weighed 0.246 gram
2.28 P.M. weighed 0.243 gram, fibers
shortened
2.36 P.M. weighed 0.240 gram
2.44 P.M. weighed 0.240 gram
2.52 P.M. weighed 0.240 gram, fibers
now much shortened,
transferred to half
strength Ringer
3.00 P.M. weighed 0.243 gram
3.08 P.M. weighed 0.245 gram
3.16 P.M. weighed 0.250 gram
3.24 P.M. weighed 0.253 gram
3.32 P.M. weighed 0.256 gram, fibers
somewhat longer than at
2.52, still very highly irri-
table
9.43 A.M. February 9, weighed 0.307
gram, fibers considerably
lengthened
1.43 P.M. weighed 0.302 gram, fibers
still considerably length-
ened and highly irritable
Temperature varied between 18° and
19°

EXPERIMENT 34

February 13, 1911

Strip of stomach muscle from bull-frog 24, weighed fresh 0.121 gram
 11.49 A.M. immersed in 0.7 per cent NaCl solution
 1.49 P.M. weighed 0.145 gram, fibers considerably lengthened
 5.38 P.M. weighed 0.152 gram, fibers shorter than at last weighing
 10.49 A.M. February 14, weighed 0.123 gram, fibers much shortened
 11.43 A.M. still slightly irritable, transferred to Ringer
 2.30 P.M. only slightly more irritable than 11.43 A.M.
 9.55 A.M. February 15, weighed 0.158 gram, fibers considerably longer than at last weighing and now fairly irritable
 Temperature varied between 19° and 22°

EXPERIMENT 36

February 13, 1911

Strip of stomach muscle from bull-frog 24, weighed fresh 0.1325 gram
 2.20 P.M. immersed in Ringer
 5.52 P.M. weighed 0.150 gram, fibers not much changed in length
 11.20 A.M. February 14, weighed 0.165 gram, fibers now considerably lengthened and highly irritable
 10.35 A.M. February 15, weighed 0.140 gram, fibers much shorter than at last weighing, and still somewhat irritable
 The temperature varied between 19° and 22°

EXPERIMENT 35

February 13, 1911

Strip of stomach muscle from bull-frog 24, weighed fresh 0.145 gram
 2.12 P.M. immersed in 0.7 per cent NaCl solution
 5.49 P.M. weighed 0.174 gram, fibers not much changed in length
 11.12 A.M. February 14, weighed 0.150 gram, fibers now much shortened
 2.33 P.M. gave no response to tetanizing current applied for one second; transferred to Ringer
 10.25 A.M. February 15, weighed 0.157 gram, fibers only slightly longer than at last weighing; gave decided though small response to tetanizing current applied for one second
 Temperature varied between 19° and 22°

EXPERIMENT 37

February 13, 1911

Strip of stomach muscle from bull-frog 24, weighed fresh 0.145 gram
 2.09 P.M. immersed in Ringer without NaHCO₃
 5.46 P.M. weighed 0.1705 gram, fibers not much changed in length
 11.09 A.M. February 14, weighed 0.180 gram, fibers now considerably lengthened and highly irritable
 Temperature varied between 20° and 22°

EXPERIMENT 38

February 15, 1911

Tendo Achillis of bull-frog 25,
weighed fresh 0.350 gram
2.15 P.M. immersed in Ringer
2.45 P.M. weighed 0.377 gram
4.51 P.M. weighed 0.410 gram
9.37 A.M. February 16, weighed 0.431
gram
Temperature varied between 18° and
21°

EXPERIMENT 39

February 15, 1911

Tendo Achillis of bull-frog 25,
weighed fresh 0.395 gram
2.23 P.M. immersed in Ringer without
 NaHCO_3 + 0.025 per cent
lactic acid
2.53 P.M. weighed 0.417 gram
4.51 P.M. weighed 0.483 gram
9.42 A.M. February 16, weighed 0.577
gram
Temperature varied between 18° and
21°

EXPERIMENT 40

February 15, 1911

Tendo Achillis of bull-frog 26,
weighed fresh 0.275 gram
2.32 P.M. immersed in 0.7 per cent
 NaCl solution
3.02 P.M. weighed 0.293 gram
4.59 P.M. weighed 0.320 gram
9.49 A.M. February 16, weighed 0.325
gram
Temperature varied between 18° and
21°

EXPERIMENT 41

February 15, 1911

Tendo Achillis of bull-frog 26,
weighed fresh 0.206 gram
2.30 P.M. immersed in distilled water
3.09 P.M. weighed 0.200 gram
5.02 P.M. weighed 0.280 gram
10.00 A.M. February 16, weighed 0.270
gram
Temperature varied between 18° and
21°

EXPERIMENT 42

February 16, 1911

Round part of tendo Achillis of bull-frog 27, weighed fresh 0.302 gram
11.02 A.M. immersed in Ringer without NaHCO_3
11.32 A.M. weighed 0.318 gram
3.02 P.M. weighed 0.338 gram
10.32 A.M. February 17, weighed 0.344 gram
4.02 P.M. weighed 0.340 gram
10.32 A.M. February 18, weighed 0.333 gram
Temperature varied between 19° and 21°

EXPERIMENT 43

February 16, 1911

Flat part of tendo Achillis of bull-frog 27, weighed fresh 0.062 gram

11.08 A.M. immersed in Ringer without NaHCO_3

11.38 A.M. weighed 0.069 gram

3.08 P.M. weighed 0.067 gram

10.38 A.M. February 17, weighed 0.068 gram

4.08 P.M. weighed 0.070 gram

10.38 A.M. February 18, weighed 0.072 gram

Temperature varied between 19° and 21°

EXPERIMENT 44

February 16, 1911

Flat part of tendo Achillis of bull-frog 27, weighed fresh 0.032 gram

11.30 A.M. immersed in Ringer without NaHCO_3 + 0.01 per cent lactic acid

12.00 M. weighed 0.0325 gram

3.30 P.M. weighed 0.033 gram

11.00 A.M. February 17, weighed 0.045 gram

4.30 P.M. weighed 0.053 gram

11.00 A.M. February 18, weighed 0.062 gram

Temperature varied between 19° and 21°

EXPERIMENT 45

February 22, 1911

Sartorius of bull-frog 28, weighed fresh 0.180 gram

3.08 P.M. immersed in 2.3 per cent alanin solution (neutral reaction); shortened somewhat and remained shortened

3.28 P.M. weighed 0.180 gram

3.48 P.M. weighed 0.180 gram

4.08 P.M. weighed 0.176 gram

4.35 P.M. no longer irritable, transferred to Ringer

4.53 P.M. quite perceptibly irritable

10.30 A.M. February 23, now quite irritable, though rigor is well started.

Temperature varied between 19° and 20°

EXPERIMENT 46

February 22, 1911

Portion of stomach muscle of bull-frog 28, weighed fresh 0.177 gram

3.18 P.M. immersed in 2.3 per cent alanin solution (neutral reaction)

3.38 P.M. weighed 0.179 gram

3.58 P.M. weighed 0.195 gram, fibers beginning to lengthen

4.18 P.M. weighed 0.208 gram, fibers now considerably lengthened and still quite irritable

10.45 A.M. February 23, weighed 0.268 gram, fibers now very much lengthened, and still somewhat irritable, alanin solution still has neutral reaction

Temperature varied between 19° and 20°

EXPERIMENT 47

February 24, 1911

Tendo Achillis of bull-frog 29, weighed fresh 0.317 gram
10.20 A.M. immersed in Ringer at room temperature (see below)
10.50 A.M. weighed 0.375 gram
11.50 A.M. weighed 0.398 gram
1.50 P.M. weighed 0.417 gram
4.50 P.M. weighed 0.422 gram
9.50 A.M. February 25, weighed 0.427 gram
Temperature varied between 20° and 22°

EXPERIMENT 48

February 24, 1911

Tendo Achillis of bull-frog 29, weighed fresh 0.367 gram
10.22 A.M. immersed in Ringer at 4
10.52 A.M. weighed 0.392 gram
11.52 A.M. weighed 0.410 gram
1.52 P.M. weighed 0.426 gram
4.52 P.M. weighed 0.437 gram
Temperature up to this point varied between 0 and 1
9.52 A.M. February 25, weighed 0.445 gram, temperature has risen to 5.5°

EXPERIMENT 49

March 3, 1911

Sartorius of bull-frog 30, weighed fresh 0.171 gram
10.23 A.M. immersed in 9 per cent cane sugar solution
10.43 A.M. weighed 0.174 gram
11.03 A.M. weighed 0.171 gram
11.23 A.M. weighed 0.168 gram
12.23 P.M. weighed 0.164 gram, still appears normal, but is entirely unirritable, transferred to Ringer
12.55 P.M. now quite irritable
2.38 P.M. still quite irritable
Temperature varied between 18° and 20°

EXPERIMENT 50

March 3, 1911

Portion of stomach muscle of bull-frog 30, weighed fresh 0.230 gram
10.20 A.M. immersed in 9 per cent cane sugar solution
10.40 A.M. weighed 0.235 gram, fibers somewhat lengthened
11.00 A.M. weighed 0.245 gram
11.20 A.M. weighed 0.250 gram
12.20 P.M. weighed 0.230 gram, fibers only slightly longer than at 10.40 A.M. still highly irritable
Temperature varied between 18° and 20°

EXPERIMENT 51

March 29, 1911

Sartorius of bull-frog 31, weighed fresh 0.186 gram
 2.19 P.M. immersed in Ringer
 3.31 P.M. weighed 0.186 gram
 3.39 P.M. weighed 0.185 gram
 3.47 P.M. weighed 0.186 gram
 3.55 P.M. weighed 0.1855 gram, transferred to double strength Ringer
 4.03 P.M. weighed 0.175 gram
 4.11 P.M. weighed 0.170 gram
 4.19 P.M. weighed 0.167 gram
 4.27 P.M. weighed 0.163 gram
 4.35 P.M. weighed 0.160 gram
 4.43 P.M. weighed 0.157 gram
 4.51 P.M. weighed 0.154 gram
 4.59 P.M. weighed 0.154 gram, still somewhat irritable
 9.31 A.M. March 30, weighed 0.166 gram, entirely unirritable, but shows little or no sign of oncoming rigor
 Temperature varied between 20° and 21°

EXPERIMENT 52

March 29, 1911

Portion of stomach muscle of bull-frog 31, weighed fresh 0.255 gram
 2.15 P.M. immersed in Ringer
 3.27 P.M. weighed 0.266 gram
 3.35 P.M. weighed 0.265 gram
 3.43 P.M. weighed 0.266 gram
 3.51 P.M. weighed 0.267 gram transferred to double strength Ringer
 3.59 P.M. weighed 0.2595 gram
 4.07 P.M. weighed 0.243 gram, fibers much shortened
 4.15 P.M. weighed 0.233 gram
 4.23 P.M. weighed 0.231 gram
 4.31 P.M. weighed 0.227 gram
 4.39 P.M. weighed 0.223 gram
 4.47 P.M. weighed 0.221 gram
 4.55 P.M. weighed 0.222 gram, fibers now very much shortened, they show a marked tendency to lengthen on stimulation;¹ transferred to Ringer.
 9.45 A.M. March 30, fibers considerably longer than at 4.55 P.M. yesterday, and quite irritable
 Temperature varied between 20° and 21°

¹ See p. 509.

EXPERIMENT 53

March 30, 1911

Portion of stomach muscle of green frog 32, weighed fresh 0.157 gram
 11.00 A.M. immersed in Ringer
 12.00 M. weighed 0.175 gram, fibers considerably lengthened
 1.00 P.M. weighed 0.185 gram
 2.00 P.M. weighed 0.187 gram
 3.00 P.M. weighed 0.190 gram
 4.00 P.M. weighed 0.191 gram
 5.00 P.M. weighed 0.189 gram
 10.00 A.M. March 31, weighed 0.195 gram, fibers still lengthened, but shortened somewhat as the result of handling and drying
 11.00 A.M. weighed 0.190 gram, fibers not much changed in length and still very highly irritable

EXPERIMENT 54

March 30, 1911

Sartorius of green frog 32, weighed fresh 0.144 gram
 11.16 A.M. immersed in Ringer
 1.10 P.M. weighed 0.138 gram
 1.18 P.M. weighed 0.137 gram
 1.26 P.M. weighed 0.136 gram
 1.34 P.M. weighed 0.136 gram
 1.42 P.M. weighed 0.137 gram
 1.50 P.M. weighed 0.136 gram, transferred to double strength Ringer
 1.58 P.M. weighed 0.127 gram
 2.06 P.M. weighed 0.123 gram
 2.14 P.M. weighed 0.121 gram
 2.22 P.M. weighed 0.1195 gram
 2.30 P.M. weighed 0.118 gram
 2.38 P.M. weighed 0.117 gram
 2.46 P.M. weighed 0.116 gram
 2.54 P.M. weighed 0.115 gram
 3.02 P.M. weighed 0.114 gram
 3.10 P.M. weighed 0.113 gram
 3.18 P.M. weighed 0.112 gram
 3.26 P.M. weighed 0.112 gram
 3.34 P.M. weighed 0.1125 gram
 3.42 P.M. weighed 0.112 gram
 3.50 P.M. weighed 0.111 gram
 3.58 P.M. weighed 0.111 gram
 4.13 P.M. weighed 0.1115 gram, still somewhat irritable, but contraction is very slow
 4.56 P.M. weighed 0.114 gram
 9.50 A.M. March 31, weighed 0.152 gram, entirely unirritable, but shows little sign of oncoming rigor; transferred to Ringer
 11.10 A.M. still entirely unirritable, little sign of oncoming rigor
 Temperature varied between 18° and 21°

EXPERIMENT 55

March 30, 1911

Portion of stomach muscle of green frog 32, weighed fresh 0.182 gram
 11.12 A.M. immersed in Ringer
 1.06 P.M. weighed 0.205 gram, fibers much lengthened
 1.14 P.M. weighed 0.202 gram, fibers much shorter than at 1.06
 1.22 P.M. weighed 0.197 gram
 1.30 P.M. weighed 0.190 gram
 1.38 P.M. weighed 0.188 gram
 1.46 P.M. weighed 0.188 gram, fibers now still shorter than at 1.14; transferred to double strength Ringer
 1.54 P.M. weighed 0.179 gram
 2.02 P.M. weighed 0.162 gram, fibers markedly further shortened
 2.10 P.M. weighed 0.151 gram
 2.18 P.M. weighed 0.148 gram
 2.26 P.M. weighed 0.141 gram
 2.34 P.M. weighed 0.141 gram
 2.42 P.M. weighed 0.140 gram
 2.50 P.M. weighed 0.141 gram
 2.58 P.M. weighed 0.142 gram
 3.06 P.M. weighed 0.142 gram
 3.14 P.M. weighed 0.1425 gram
 3.22 P.M. weighed 0.143 gram
 3.30 P.M. weighed 0.144 gram
 3.38 P.M. weighed 0.145 gram
 3.46 P.M. weighed 0.145 gram
 3.54 P.M. weighed 0.146 gram
 4.09 P.M. weighed 0.148 gram, fibers still shortened; they show a marked tendency to lengthen on stimulation after a latent period of about 20 seconds¹
 4.27 P.M. transferred to Ringer
 9.00 A.M. March 31, fibers somewhat longer than at 4.27 yesterday and highly irritable
 Temperature varied between 18° and 21°

¹ See p. 509

EXPERIMENT 56

April 3, 1911

Sartorius of bull-frog 33, weighed fresh 0.173 gram
 10.33 A.M. immersed in Ringer
 1.42 P.M. weighed 0.160 gram
 1.50 P.M. weighed 0.159 gram
 1.58 P.M. weighed 0.158 gram
 2.06 P.M. weighed 0.158 gram
 2.14 P.M. weighed 0.158 gram, immersed in half strength Ringer
 2.22 P.M. weighed 0.169 gram
 2.30 P.M. weighed 0.175 gram
 2.38 P.M. weighed 0.180 gram, still very highly irritable
 2.46 P.M. weighed 0.184 gram
 2.54 P.M. weighed 0.187 gram
 3.02 P.M. weighed 0.1905 gram
 3.10 P.M. weighed 0.1905 gram, still very highly irritable
 3.18 P.M. weighed 0.193 gram
 3.26 P.M. weighed 0.195 gram
 3.34 P.M. weighed 0.196 gram
 3.42 P.M. weighed 0.198 gram
 3.50 P.M. weighed 0.199 gram
 3.58 P.M. weighed 0.199 gram
 4.06 P.M. weighed 0.199 gram, still very highly irritable
 4.50 P.M. weighed 0.205 gram, still very highly irritable
 10.22 A.M. April 4, weighed 0.202 gram, fibers somewhat shortened, but still highly irritable
 Temperature varied between 15° and 18°

EXPERIMENT 57

April 3, 1911

Portion of stomach of bull-frog 33, weighed fresh 0.262 gram
 10.37 A.M. immersed in Ringer
 1.46 P.M. weighed 0.284 gram, fibers not much changed in length
 1.54 P.M. weighed 0.277 gram, fibers slightly shorter than at 1.46
 2.02 P.M. weighed 0.270 gram
 2.10 P.M. weighed 0.267 gram
 2.18 P.M. weighed 0.262 gram, fibers slightly shorter than at 1.54, immersed in half strength Ringer
 2.26 P.M. weighed 0.262 gram
 2.34 P.M. weighed 0.260 gram, fibers rather shorter than at 2.18
 2.42 P.M. weighed 0.264 gram
 2.50 P.M. weighed 0.266 gram, fibers not much changed in length
 2.58 P.M. weighed 0.268 gram
 3.06 P.M. weighed 0.272 gram, fibers now somewhat longer than at 2.50
 3.14 P.M. weighed 0.271 gram
 3.22 P.M. weighed 0.271 gram
 3.30 P.M. weighed 0.273 gram
 3.38 P.M. weighed 0.275 gram
 3.46 P.M. weighed 0.274 gram
 3.54 P.M. weighed 0.278 gram
 4.02 P.M. weighed 0.278 gram
 4.10 P.M. weighed 0.278 gram, fibers not much changed in length since 3.06
 4.54 P.M. weighed 0.288 gram, fibers slightly longer than at 4.10
 10.26 A.M. April 4, weighed 0.311 gram, fibers were longer than at 4.54 P.M. yesterday, but shortened as result of handling and drying; muscle still quite irritable
 Temperature varied between 15° and 18°

EXPERIMENT 58

April 28, 1911

Portions of stomach muscle¹ of bullfrogs Nos. 34, 35, 36, 37, 38, 39, 40, 41, 42 and 43, weighed fresh 6.3422 grams

This portion of muscle was analyzed for potassium and sodium and found to contain 0.3437 per cent potassium and 0.0804 per cent sodium

¹ Each piece of stomach muscle was cut into a cardiac and pyloric half, and the portions of muscle used in this experiment and in Experiment 59 contained equal numbers of cardiac and pyloric halves

EXPERIMENT 59

April 28, 1911

Portions of stomach muscle² of bullfrogs Nos. 34, 35, 36, 37, 38, 39, 40, 41, 42 and 43, weighed fresh 6.3048 grams

This portion of muscle was placed for five hours in 7.5 per cent cane sugar solution, which was frequently stirred and changed three times. In all about 200 cc. of the sugar solution were used. At the end of the five hours the muscle weighed 6.3091 grams, and was still highly irritable. It was then analyzed for potassium and sodium and found to contain 0.3299 per cent potassium and 0.0460 per cent sodium.

The fresh muscle contained 0.3437 per cent potassium and 0.0804 per cent sodium. Therefore, 0.3437-0.3299 or 0.0138 per cent potassium, and 0.0804-0.0460 or 0.0344 per cent sodium may be supposed to have diffused out into the sugar solution.

Temperature varied between 22° and 25°

² See footnote to Experiment 58.

EXPERIMENT 60

May 22, 1911

Portion of stomach muscle of bullfrog 44, weighed fresh 0.273 gram.

10.45 A.M. immersed in 7.5 per cent cane sugar solution

11.00 A.M. weighed 0.310 gram, fibers somewhat lengthened

11.45 A.M. weighed 0.327 gram, fibers considerably lengthened

1.45 P.M. weighed 0.334 gram, fibers still considerably lengthened, transferred to fresh sugar solution

3.45 P.M. weighed 0.391 gram, fibers considerably longer than at 1.45 and still fairly irritable

Temperature varied between 26° and 29°

EXPERIMENT 61

May 22, 1911

Portion of stomach muscle of bullfrog 44, weighed fresh 0.208 gram

10.49 A.M. immersed in mixture made up of 1 part Ringer and 19 parts 7.5 per cent cane sugar solution

11.04 A.M. weighed 0.214 gram, fibers have not changed in length

11.49 A.M. weighed 0.210 gram, fibers now somewhat shortened

1.49 P.M. weighed 0.205 gram, fibers still shortened

3.49 P.M. weighed 0.214 gram, fibers still shortened, and fairly irritable

Temperature varied between 26° and 29°

EXPERIMENT 62

May 23, 1911

Portions of stomach muscle of bullfrogs Nos. 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, weighed fresh 5.4701 grams

This muscle was kept for five hours, 30 minutes in a moist chamber at 29° and then dried at between 100° and 110°

Its final dry weight was 0.9748 gram

EXPERIMENT 63

May 23, 1911

Portions of stomach muscle of bullfrogs Nos. 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, and 60, weighed fresh 5.3976 grams

This muscle was placed for five hours, 50 minutes in 7.5 per cent cane sugar solution at 29°. The sugar solution was stirred and changed frequently, in all about 700 cc. of it were used. The muscle was still fairly irritable at the end of its stay in the sugar solution and weighed 6.1854 grams. It was dried at between 100° and 110° and its final dry weight was 1.1422 grams

EXPERIMENT 64

April 10, 1912

Sartorius of leopard frog 61, weighed fresh 0.182 gram

1.00 P.M. immersed in 0.9 per cent KCl solution

1.30 P.M. weighed 0.194 gram, fibers not shortened, but entirely unirritable

2.00 P.M. weighed 0.216 gram

2.30 P.M. weighed 0.229 gram, fibers rather longer than originally

3.00 P.M. weighed 0.252 gram, fibers now very slightly shorter than originally

3.30 P.M. still very slightly shorter than originally and rather stiff and gelatinous; transferred to Ringer

4.10 still entirely unirritable

11.15 A.M. April 11, weighed 0.223 gram, now quite irritable through whole extent

Temperature varied between 18° and 19°

EXPERIMENT 65

April 10, 1912

Portion of stomach muscle of leopard frog 61, weighed fresh 0.161 gram

1.10 P.M. immersed in 0.9 per cent KCl solution

1.40 P.M. weighed 0.156 gram, fibers little changed in length

2.10 P.M. weighed 0.158 gram, fibers slightly shortened

2.40 P.M. weighed 0.162 gram, fibers still slightly shortened

3.10 P.M. weighed 0.165 gram, fibers still slightly shortened

3.40 P.M. muscle still highly irritable

11.25 A.M. April 11, weighed 0.234 gram, fibers now considerably lengthened and still fairly irritable

Temperature varied between 18° and 19°

EXPERIMENT 66

April 10, 1912

Sartorius of leopard frog 61, weighed fresh 0.192 gram
1.05 P.M. immersed in 1.3 per cent K_2HPO_4 solution
1.35 P.M. weighed 0.198 gram, fibers not shortened, but entirely unirritable
2.05 P.M. weighed 0.201 gram
2.35 P.M. weighed 0.203 gram
3.05 P.M. weighed 0.206 gram
3.35 P.M. fibers have about same length as originally, transferred to Ringer
4.10 P.M. now quite irritable.¹
11.23 A.M. April 11th, weighed 0.200 gram, now highly irritable
Temperature varied between 18° and 19°

¹ Ringer solution was not agitated between 3.35 and 4.10

EXPERIMENT 68

April 10, 1912

Sartorius of leopard frog 62, weighed fresh 0.140 gram
1.20 P.M. immersed in 1 per cent $NaC_2H_3O_2$ solution
1.50 P.M. weighed 0.138 gram, fibers markedly shortened, but still somewhat irritable
2.20 P.M. weighed 0.145 gram
2.50 P.M. weighed 0.150 gram
3.20 P.M. weighed 0.150 gram, fibers still shortened and somewhat irritable, have all along been twitching more or less rhythmically in the solution
12.05 P.M. April 11, weighed 0.142 gram, fibers still markedly shortened and entirely unirritable; transferred to Ringer
1.40 P.M. now somewhat irritable
Temperature varied between 18° and 19°

EXPERIMENT 67

April 10, 1912

Portion of stomach muscle of leopard frog 61, weighed fresh 0.139 gram
1.15 P.M. immersed in 1.3 per cent K_2HPO_4 solution
1.45 P.M. weighed 0.141 gram, fibers little changed in length
2.15 P.M. weighed 0.141 gram, fibers slightly lengthened
2.45 P.M. weighed 0.145 gram, fibers still slightly lengthened
3.15 P.M. weighed 0.146 gram, fibers still slightly lengthened
3.45 P.M. still highly irritable
11.45 A.M. April 11, weighed 0.167 gram, fibers somewhat further lengthened, and still fairly irritable, though rather less so than those of companion of Experiment 65 which had been in 0.9 per cent KCl solution
Temperature varied between 18° and 19°

EXPERIMENT 69

April 10, 1912

Portion of stomach muscle of leopard frog 62, weighed fresh 0.157 gram
1.25 P.M. immersed in 1 per cent $NaC_2H_3O_2$ solution
1.55 P.M. weighed 0.158 gram, fibers slightly lengthened
2.25 P.M. weighed 0.161 gram, fibers somewhat further lengthened
2.55 P.M. weighed 0.162 gram, fibers have about same length as at last weighing
3.25 P.M. weighed 0.160 gram, fibers slightly longer than at 2.55
3.55 P.M. still quite irritable
11.55 A.M. April 11, weighed 0.165 gram, fibers somewhat longer than at 3.25 P.M. yesterday, and still quite irritable
Temperature varied between 18° and 19°

EXPERIMENT 70

April 16, 1912

Sartorius from leopard frog 63¹
 weighed fresh 0.141 gram
 10.45 A.M. immersed in 12 per cent
 cane sugar solution
 11.15 A.M. weighed 0.115 gram, fibers
 about same length as orig-
 inally, no sign of rigor
 11.45 A.M. weighed 0.112 gram
 12.15 P.M. weighed 0.115 gram
 12.45 P.M. weighed 0.114 gram, contracts
 slowly and remains con-
 tracted in neighborhood
 of stimulation; trans-
 ferred to Ringer
 3.00 P.M. twitches now in usual way
 on stimulation
 4.20 P.M. now somewhat more irrita-
 ble than at 3.00

Temperature varied between 20° and
 21°

¹ This frog's tissues were somewhat
 oedematous.

EXPERIMENT 71

April 16, 1912

Stomach muscle from leopard frog
 63² weighed fresh 0.159 gram
 10.50 A.M. immersed in 12 per cent cane
 sugar solution
 11.20 A.M. weighed 0.161 gram, fibers
 slightly shortened
 11.50 A.M. weighed 0.183 gram, fibers
 not much changed in
 length
 12.20 P.M. weighed 0.214 gram, fibers
 slightly longer than at
 11.50 A.M.
 12.50 P.M. weighed 0.233 gram, fibers
 now considerably length-
 ened, especially at cardiac
 end
 4.50 P.M. weighed 0.235 gram, fibers
 now much lengthened es-
 pecially at cardiac end;
 cardiac end no longer irri-
 table; pyloric end very
 slightly so
 5.25 P.M. transferred to Ringer
 10.30 A.M. April 17, all parts of muscle
 now lengthened and unir-
 ritable

Temperature varied between 19° and
 21°

² This frog's tissues were somewhat
 oedematous

EXPERIMENT 72

April 16, 1912

Sartorius of leopard frog 6,¹ weighed fresh 0.139 gram
 11.00 A.M. immersed in 0.9 per cent KCl solution
 11.30 A.M. weighed 0.157 gram
 12.00 M. weighed 0.178 gram
 12.30 P.M. weighed 0.182 gram
 1.00 P.M. weighed 0.195 gram
 5.00 P.M. weighed 0.273 gram, fibers not shortened, entirely unirritable, temperature up to this point had varied between 20° and 21°; muscle transferred to Ringer at 12°
 9.30 A.M. April 17, still entirely unirritable, fibers little shortened, temperature since 5.00 P.M. April 16 has remained at 12°, now allowed to rise to 19°
 10.30 A.M. fibers now considerably shortened
 4.10 P.M. fibers still shortened and entirely unirritable, temperature has remained since 10.30 A.M. at 19°

¹ This frog's tissues were somewhat oedematous

EXPERIMENT 73

April 16, 1912

Stomach muscle from leopard frog 63,² weighed fresh 0.241 gram
 11.10 A.M. immersed in 0.9 per cent KCl solution
 11.40 A.M. weighed 0.224 gram, fibers somewhat shortened
 12.10 P.M. weighed 0.227 gram, fibers still shortened
 12.40 P.M. weighed 0.237 gram, fibers still shortened
 1.10 P.M. weighed 0.249 gram, fibers very slightly longer than at last weighing
 4.10 P.M. weighed 0.283 gram, fibers somewhat longer than at 1.10
 11.10 A.M. April 17, weighed 0.372 gram, fibers now much lengthened, still somewhat irritable, though latent period is long
 Temperature varied between 19° and 21°
² This frog's tissues were somewhat oedematous

EXPERIMENT 74

April 18, 1912

Sartorius from small bull-frog 64, weighed fresh 0.195 gram
 10.20 A.M. immersed in 0.9 per cent KCl solution
 11.20 A.M. weighed 0.213 gram, fibers not changed in length
 12.20 P.M. weighed 0.235 gram, muscle still has original length and is rather stiff and gelatinous
 1.20 P.M. weighed 0.255 gram, entirely unirritable, transferred to Ringer
 2.20 P.M. still entirely unirritable; fibers not changed in length
 3.20 P.M. now somewhat irritable, particularly at thin lower end; Ringer solution has been agitated a good deal between 1.20 and 3.20
 4.20 P.M. now quite irritable
 9.40 A.M. April 19, weighed 0.192 gram, now highly irritable through whole extent to mechanical as well as electrical stimulation, not shortened and shows no sign of rigor
 Temperature throughout this experiment was 16°

EXPERIMENT 75

April 18, 1912

Sartorius from small bull-frog 64, weighed fresh 0.195 gram

11.30 A.M. immersed in 3.95 per cent dextrose solution

12.00 M. weighed 0.198 gram, fibers slightly shortened

12.30 P.M. weighed 0.219 gram

1.00 P.M. weighed 0.222 gram

1.30 P.M. weighed 0.223 gram

3.30 P.M. weighed 0.221 gram, shows barely perceptible irritability; transferred to Ringer

4.30 P.M. now fairly irritable¹

9.30 A.M. April 19, now quite irritable

Temperature throughout this experiment remained at 16°

¹ Ringer solution was not agitated between 3.30 and 4.30

EXPERIMENT 76

April 18, 1912

Stomach muscle from small bull-frog 64, weighed fresh 0.263 gram

11.35 A.M. immersed in 3.95 per cent dextrose solution

12.05 P.M. weighed 0.270 gram, fibers somewhat lengthened

12.35 P.M. weighed 0.310 gram, fibers somewhat further lengthened

1.05 P.M. weighed 0.345 gram, fibers markedly further lengthened

1.35 P.M. weighed 0.392 gram, fibers now very much lengthened

3.35 P.M. weighed 0.480 gram, fibers now enormously lengthened

3.55 P.M. still quite irritable

10.05 A.M. April 19, weighed 0.480 gram, fibers have about same length as at 3.35 P.M. yesterday, still slightly irritable

Temperature throughout this experiment remained at 16°

EXPERIMENT 77

April 18, 1912

Sartorius from small bull-frog 65, weighed fresh 0.172 gram

10.25 A.M. immersed in 0.9 per cent KCl solution at 4°

11.25 A.M. weighed 0.188 gram, fibers somewhat shortened

12.25 P.M. weighed 0.204 gram, fibers now have original length, muscle somewhat stiff and gelatinous

1.25 P.M. weighed 0.215 gram

4.25 P.M. weighed 0.258 gram, entirely unirritable, temperature of KCl solution has varied between 2° and 8°, muscle transferred to Ringer at 9°

5.25 P.M. temperature of Ringer is 11°

9.30 A.M. April 19, weighed 0.196 gram, now quite irritable through whole extent, not shortened and shows no sign of rigor. Temperature of Ringer solution has risen gradually since 5.25 P.M. yesterday to 16°

EXPERIMENT 78

April 18, 1912

Sartorius from small bull-frog 65, weighed fresh 0.185 gram
 11.40 A.M. immersed in 7.5 per cent lactose solution
 12.10 P.M. weighed 0.186 gram, fibers not changed in length
 12.40 P.M. weighed 0.191 gram
 1.10 P.M. weighed 0.200 gram
 1.40 P.M. weighed 0.199 gram
 3.40 P.M. weighed 0.193 gram, shows barely perceptible irritability; transferred to Ringer
 4.30 P.M. now fairly irritable¹
 9.30 A.M. April 19, now quite irritable
 Temperature remained throughout this experiment at 16°

¹ Ringer solution was not agitated between 3.40 and 4.30

EXPERIMENT 79

April 18, 1912

Stomach muscle from small bull-frog 65, weighed fresh 0.192 gram
 11.45 A.M. immersed in 7.5 per cent lactose solution
 12.15 P.M. weighed 0.201 gram, fibers somewhat lengthened
 12.45 P.M. weighed 0.228 gram, fibers somewhat further lengthened
 1.15 P.M. weighed 0.252 gram, fibers markedly further lengthened, especially at cardiac end
 1.45 P.M. weighed 0.282 gram, fibers now very much lengthened
 3.45 P.M. weighed 0.306 gram, fibers somewhat longer than at 1.45
 4.10 P.M. still quite irritable
 10.15 A.M. April 19, weighed 0.256 gram, fibers a little shorter than at 3.45 P.M. yesterday, cardiac end no longer irritable, pyloric end very slightly so
 Temperature remained throughout this experiment at 16°

CONCERNING NEGATIVE PHOTOTROPISM IN DAPHNIA PULEX

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ONE FIGURE

It has been shown by Loeb¹ that the rays of a Heraeus mercury arc cause *Balanus* larvae and *Daphnia* to become negatively phototropic. This effect is due principally to the ultra-violet rays given off by the mercury lamp, because if a plate of ordinary window-glass be interposed between the light and the dish containing the animals, thus cutting off the greater part of the ultra-violet rays, the negative effect of the light is much diminished.

Before making further experiments along this line, I wished to determine at what point in the spectrum one thickness of window-glass cuts off the ultra-violet light effectively. To this end spectrophotographs were taken with and without the glass plate interposed, exposure twenty seconds. I am greatly indebted to Professor Minor, of the Department of Physics, for making the spectrophotographs and identifying the lines. Figure 1 at the top shows a spectrophotograph of the mercury arc used; and below a photograph of the same with the glass plate interposed between the arc and spectroscop.

It is apparent that a pair of lines in the ultra-violet (lines of wave length 3341 Å. u.² and 3390 Å. u.) pass through the glass plate only slightly impaired. Therefore, the much stronger negative effect of the light which does not pass through a glass plate must be due to the rays of wave length shorter than 3341 Å. u.

¹ Loeb, J. Pflüger's Archiv, Bd. 115, S. 576.

² Ångström units.

The material used in the following experiments was *Daphnia pulex*, kindly identified for me by Professor Kofoed of the Department of Zoölogy. In all of the experiments the animals were put into a finger bowl with 20 cc. of tap water, and set at a distance of 1 foot from the mercury arc. Under such conditions all of the animals form a complete negative collection in twenty to thirty seconds. The *Daphnia* never move toward the light but always away even in the first second of exposure.

If the finger bowl containing the *Daphnia* be covered by a plate of ordinary window-glass, thus cutting off the light of wave length shorter than 3341 Å. u., the results are not so uniform. In some cases there is an irregular wandering away from the light, but never a negative collection. Usually even after one and one-half hours' continuous exposure, the *Daphnia* are still scattered about the dish. Sometimes they even form a positive collection.

That the action of the light of wave length shorter than 3341 Å. u. is specific for negative phototropism of *Daphnia* may be shown by a simple experiment. First cause the animals to form a negative collection in an open dish, then cover with a glass plate without otherwise interrupting the light. The negative collection then begins to break up and the animals tend to scatter evenly about the dish. In some cases the greater part of them wander about on the side away from the light, in other cases they form a weak positive collection.

From such experiments we must conclude (1) that light of wave length 3341 Å. u. or longer is not effective in causing negative phototropism of *Daphnia pulex*, while light of shorter wave length causes them to become negative instantly; (2) that the effect of ultra-violet light of wave length shorter than 3341 Å. u. is apparent only during the time of action of such light, and ceases to exert an effect almost instantly upon this light being cut off.

Furthermore, I found that the negative effect of ultra-violet light disappears when acids, especially CO_2 , are added in small quantities to the water containing the *Daphnia* while the latter are undergoing exposure to the ultra-violet light. If the negative

THE COMPARATIVE EFFICIENCY OF WEAK AND STRONG BASES IN ARTIFICIAL PARTHENOGENESIS

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In 1905 the writer found that it is possible to induce artificial parthenogenesis (membrane formation) in the sea urchin by weak acids, such as the monobasic fatty acids or CO_2 , but not at all or only unsatisfactorily by the strong acids, such as HCl , H_2SO_4 , oxalic acid, and others. He suggested that this paradoxical behavior was due to the fact that only those acids which diffuse easily into the egg were able to cause membrane formation.¹ This assumption was supported by the observation that there existed an analogy between the relative physiological efficiency of various organic acids and their corresponding alcohols.

This paper intends to show that the weak base NH_4OH is much more efficient in the production of artificial parthenogenesis than the strong bases NaOH , KOH , and tetraethylammoniumhydroxide. The writer found in 1907 that it is possible to substitute bases for acids in the process of artificial parthenogenesis with this difference, that the eggs had to be exposed to the alkaline solution for a considerably longer period than to the acid solution in order to cause them to develop.² The eggs of *Strongylocentrotus* could be caused to develop by putting them for nearly three hours into a mixture of 50 cc. in $\frac{2}{3}$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 0.5 or 1.0 cc. $\frac{2}{3}$ NaOH . When such

¹ Loeb, University of California Publications, vol. 2, p. 113, 1905. Also in "Die chemische Entwicklungserregung des tierischen Eies," p. 100, Berlin, 1909.

² Loeb, Ueber die allgemeinen Methoden der künstlichen Parthenogenese, Pflüger's Archiv, Bd. 118, p. 572, 1907.

eggs were then transferred for from thirty-five to forty minutes to a hypertonic solution (50 cc. sea water + 8 cc. $2\frac{1}{2}$ m NaCl + KCl + CaCl_2) a number of them formed membranes and the eggs developed afterward into swimming larvae.

Recently O. Warburg³ showed that NH_4OH diffuses rapidly into the sea urchin egg while NaOH does not, and this observation was confirmed and enlarged by Harvey.⁴ This suggested the possibility that, as in the case of acids, weak bases might be found to be more effective in producing artificial parthenogenesis than strong bases.

1. Method

In order to obtain comparable results the bases had to be added to a neutral solution instead of to sea water. An m/2 solution of (NaCl + CaCl_2 + KCl) in the usual proportion was used⁵ for this purpose. Before the eggs were put into this solution they were freed from sea water by repeated washing in a solution of the same constitution and concentration. From the alkaline solution the eggs were transferred directly into the neutral hypertonic solution. The latter consisted of 50 cc. m/2 (NaCl + CaCl_2 + KCl) + 8 cc. $2\frac{1}{2}$ m of the same mixture. From the hypertonic solution the eggs were transferred to normal sea water. They often showed a tendency to stick to the glass. This was overcome by preventing them from settling for about five minutes through gentle agitation.

2. Comparison of the efficiency of NH_4OH and KOH

To 50 cc. m/2 (NaCl + KCl + CaCl_2), 0.3 cc. $\frac{N}{10}$ NH_4OH , and 0.3 cc. $\frac{N}{10}$ KOH were added respectively. Unfertilized eggs of *Arbacia* were left in these solutions for six, twelve, twenty-four, forty-two, and sixty-one minutes. Then they were transferred for fifteen minutes into the neutral hypertonic solution,

³ O. Warburg, *Zeitsch. f. physiolog. Chem.*, Bd. 66, p. 305, 1910.

⁴ Harvey, *Jour. Exper. Zool.*, vol. 10, p. 507, 1911.

⁵ This proportion is as follows: 100 cc. m/2 NaCl + 2.2 cc. m/2 KCl + 1.5 cc. m/2 CaCl_2 .

namely, 50 cc. m. 2 (NaCl + KCl + CaCl₂) + 8 cc. 2½ m (NaCl + KCl + CaCl₂). From the hypertonic solution they were transferred into normal sea water. The temperature varied but little from 22°C. The results of the experiment follow.

a. *Eggs six minutes in the alkaline solution.* Some of the eggs which had been in NH₄OH for six minutes developed as far as the two or even the four cell stage, but no further. The blastomeres of the segmented eggs fell apart. No larvae were formed and the majority of the eggs remained unaltered. None of the eggs that had been in KOH for six minutes segmented; all remained unaltered. The eggs which did not segment had no membranes.

b. *Eggs twelve minutes in alkali.* A large percentage of the eggs that had been twelve minutes in the NH₄OH formed membranes and segmented, and a few of these developed into larvae. The eggs which had not formed membranes remained unsegmented and intact.

The eggs which had been for twelve minutes in KOH formed no membranes and did not segment or develop into larvae. A few showed amoeboid changes preceding a possible cell division. Practically all the eggs were intact on the following day.

c. *Eggs twenty-four minutes in alkali.* The eggs that had been in NH₄OH for twenty-four minutes practically all formed membranes, segmented normally to a large extent, and formed larvae. Many of the latter reached the pluteus stage, and swam at the surface of the dish.

Of the eggs that had been twenty-four minutes in KOH about 10 per cent formed membranes and began to segment, but did not go beyond the first stages of segmentation. Ninety per cent of the eggs formed no membranes, did not segment, and remained unaltered. We shall see later that such eggs, upon the addition of sperm, will develop into normal larvae, thus indicating that the treatment with KOH did not affect them.

d. *Eggs forty-two minutes in alkali.* About 90 per cent of the eggs that had been in NH₄OH for forty-two minutes formed membranes, segmented and developed into swimming larvae. Not so many reached the pluteus stage as in the previous lot.

Only a small percentage of the eggs that had been in KOH for forty-two minutes formed membranes and segmented, and only a few of these developed into swimming larvae.

e. Eggs sixty minutes in alkali. The eggs that had been in NH_4OH for sixty minutes formed membranes. Some began to segment but the majority disintegrated without reaching the larval stage.

A considerable percentage of the eggs treated with KOH for sixty minutes began to segment, but most of them disintegrated before they reached the blastula stage. The rest of the eggs remained intact.

We may summarize the result of this experiment by saying that practically all the eggs that had been treated with a 3/5000 N solution of NH_4OH for twenty-four minutes and were then put into a neutral hypertonic solution for fifteen minutes developed into larvae, this development being normal in a large number of cases. The eggs, however, that were treated with a 3/5000 N solution of KOH for twenty-four minutes and then put into a neutral hypertonic solution for fifteen minutes remained practically unaltered.

3. Comparison of the efficiency of NH_4OH , NaOH and tetraethylammoniumhydroxide

To three solutions of 50 cc. m/2 ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) were added 0.3 cc. $\frac{N}{10}$ NH_4OH , 0.3 cc. $\frac{N}{10}$ NaOH, and 0.3 cc. $\frac{N}{6}$ tetraethylammoniumhydroxide respectively. Unfertilized eggs of *Arbacia* were put into these solutions for twenty-six minutes and were then transferred directly into a neutral hypertonic solution, namely 50 cc. m/2 ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 8 cc. 2½ m. ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$). They remained here for fifteen minutes and were then transferred into normal sea water.

Practically all the eggs that had been in 0.3 cc. NH_4OH for twenty-six minutes developed into larvae (about as quickly as the larvae from fertilized eggs); the eggs that had been in 0.3 cc. NaOH, or in 0.3 cc. tetraethylammoniumhydroxide for twenty-six minutes remained practically intact. Only a few

eggs segmented, and only after a long search was it possible to find a swimming larva on the following day.

These experiments, which were repeated a number of times, indicate that the weak base NH_4OH is much more efficient in the causation of artificial parthenogenesis than the strong bases NaOH , KOH , and tetraethylammoniumhydroxide.

4. *Action of alkalis alone, without the action of hypertonic solution*

0.3 cc. $\frac{\text{N}}{10}$ NH_4OH , 0.3 cc. $\frac{\text{N}}{10}$ NaOH , and 0.3 cc. $\frac{\text{N}}{10}$ tetraethylammoniumhydroxide were added to 50 cc. $\text{m}/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) respectively. Unfertilized eggs of *Arbacia* were put into these three solutions for forty-two minutes and then transferred to normal sea water. All of the eggs that had been in the solution containing the NH_4OH segmented in a rather amoeboid way into two or four cells, after which the cells fell part and disintegrated. All of the eggs that had been in 50 cc. $\text{m}/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 0.3 cc. $\frac{\text{N}}{10}$ NaOH for forty-two minutes remained practically intact and the same was true for the eggs that had been in the tetraethylammoniumhydroxide for forty-two minutes. In order to make sure that they did not only appear normal but were normal, sperm was added to these eggs the next morning. All the eggs that had been in NaOH , and in tetraethylammoniumhydroxide, segmented normally and developed into normal embryos.

In this experiment part of the eggs were submitted for fifteen minutes to the action of the neutral hypertonic solution after they had been treated with alkali. The eggs that had been in NH_4OH developed into larvae, the others did not. It is obvious that the changes leading to parthenogenetic development are brought about considerably more rapidly by NH_4OH than by the strong bases.

All this is in complete analogy with the action of acids in artificial parthenogenesis. Only the acid or alkali which enters the egg can act, and since NH_4OH enters much more rapidly than the strong bases, the weak base NH_4OH is more efficient than the strong bases.

5. Oxidation and action of alkali in artificial parthenogenesis

In former papers the writer had shown that the parthenogenetic as well as the destructive action of KOH and NaOH upon cells can be retarded or suppressed through the removal of oxygen or the addition of a few drops of KCN.⁶ It was our intention to find out whether the action of NH_4OH in artificial parthenogenesis could also be suppressed by KCN. This is indeed the case. Two solutions of 50 cc. $\text{m}/2$ ($\text{NaCl} + \text{CaCl}_2 + \text{KCl}$) + 0.3 cc. $\frac{\text{N}}{10}$ NH_4OH were prepared. To one of these were added five drops of a 0.1 per cent solution of KCN. Unfertilized eggs of *Arbacia* were put into these solutions for forty-five minutes and then transferred to sea water. The eggs which had been in the solution containing KCN remained absolutely intact and unaltered. The next morning sperm was added and all segmented regularly, developing into perfectly normal larvae. The eggs, however, which had been in the solution not containing KCN began to segment and in a few hours disintegrated completely.

If the eggs remain for a number of hours in a mixture of 50 cc. $\text{m}/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 0.3 cc. $\frac{\text{N}}{10}$ NH_4OH + 5 drops of 0.1 per cent KCN they remain intact, but when put back into normal sea water they soon segment in an irregular way and disintegrate. This is in agreement with the well known fact that the amount of KCN added in this case only retards the oxidations but does not suppress them entirely.

These experiments throw a light upon the localization of oxidations in the cell. Warburg pointed out that the increase of the rate of oxidations in the egg by NaOH can only be ascribed to a surface action, since the NaOH does not noticeably diffuse into the egg. Wasteneys and I found that the weak base NH_4OH accelerates the rate of oxidations about one-half as much as the strong base NaOH.⁷ The fact that NH_4OH raises the rate of oxidations much more than should be expected

⁶ Pflüger's Archiv, Bd. 118, p. 30, 1907, and "Die chemische Entwicklungserregung des tierischen Eies, p. 118.

⁷ Loeb and Wasteneys, Biochem. Zeitsch., Bd. 37, p. 410, 1911.

from its low degree of dissociation becomes intelligible if we assume that the NH_4OH which diffuses into the egg influences the rate of oxidations more strongly than the alkali which acts merely on the surface of the egg. On this assumption the external surface of the egg is neither the only nor perhaps the main seat of oxidation, but oxidations occur also in the interior of the egg.

Another fact which would agree with such a view is the following: The egg of the Californian sea urchin, *Strongylocentrotus purpuratus*, cannot develop in an acid or in a neutral solution. It suffices, however, to add a small amount of neutral red to the salt solution to start the development. Neutral red is a much weaker base than NH_4OH and diffuses rapidly into the egg. It is not well conceivable that neutral red could accelerate the oxidations in the sea urchin egg except on the assumption that NaOH acts only or at least mainly on the surface, while neutral red acts in addition inside the egg. Since in this case the action of the alkali consists also in an acceleration of the rate of the oxidations this would also point towards the probability that the external surface is not the only seat of oxidations in the cell.

Finally, it should not be overlooked that the strong bases NaOH or KOH have also a small parthenogenetic effect. In view of the experiments mentioned in this paper this fact suggests the possibility that the strong bases diffuse into the egg to a slight extent, or at least into its cortical layer. It is probable that for this diffusion only the undissociated molecules of these bases are to be considered. The fact that the strong bases do not change the color of the neutral red contained in the egg does not contradict such an assumption, if we suppose that the base acts in the egg through salt formation with an acid constituent of the egg, e.g., an acid protein.

6. Membrane formation by alkali

In his former experiments on the parthenogenetic action of alkalies in *Strongylocentrotus* the writer pointed out that the

eggs which are induced to develop under the influence of KOH form a membrane, but that this membrane formation takes place, as a rule, not in the alkaline solution but in the hypertonic solution. The egg of *Arbacia* does not form as distinct a membrane under the influence of alkali as does the egg of *Strongylocentrotus*. In *Arbacia*, as a rule, only a fine gelatinous layer is formed at the surface of the egg through the agencies of artificial parthenogenesis.

When the eggs of *Arbacia* are put for twenty-five minutes into a mixture of 50 cc. $m/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 0.3 cc. $\frac{N}{10}$ NH_4OH and then transferred to a neutral hypertonic solution for about fifteen minutes, (at a temperature of about 22°C .), as a rule a large percentage, if not all the eggs, will develop into larvae and it will be found that the eggs which develop possess a membrane. The membrane in this case is not formed while the eggs are in the alkaline solution but while they are in the hypertonic solution. When the eggs are left in the alkaline solution much longer than twenty-five minutes membranes may be formed in the latter.

The following observation is of interest. Eggs were put for forty-five minutes into a mixture of 50 cc. $m/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 0.3 cc. $\frac{N}{10}$ NH_4OH + 5 drops 0.1 per cent KCN. From here they were transferred for fifteen minutes into the neutral hypertonic solution and then into sea water. All these eggs formed membranes but perished by cytolysis, the pigment gathering as a rule in one or more spots of the egg. The reader will remember that such eggs when put directly from the alkaline solution with KCN into sea water (without undergoing a treatment with the neutral hypertonic solution) form no membrane, and remain intact, developing normally if later on fertilized by sperm.

Why then does the treatment of such eggs for fifteen minutes with the hypertonic solution kill them? The experiment seems to indicate that the NH_4OH has two effects, one of which consists in inducing the process of membrane formation. This process in this experiment was not inhibited through the presence of KCN in the NH_4OH solution. In former papers the

writer has shown that the mere membrane formation leads to the death of the sea urchin egg unless the latter is put into the hypertonic solution for a sufficiently long time. If the eggs do not remain in the hypertonic solution a sufficient period of time after the artificial membrane formation they will perish. Such was the case in this experiment. In order to make this clear another set of experiments must be discussed.

7. *Variation of the time of exposure to the hypertonic solution*

In the experiments thus far mentioned the eggs of *Arbacia* were always exposed to the neutral hypertonic solution for fifteen minutes at a temperature of about 22°C. When the eggs had previously been treated for about twenty-five minutes with a mixture of 50 cc. $m/2$ (NaCl + KCl + $CaCl_2$) + 0.3 cc. $\frac{N}{10}$ NH_4OH the exposure of fifteen minutes to a neutral hypertonic solution as a rule sufficed to cause all or many of the eggs to develop. If the eggs remain in the hypertonic solution for a longer period, they develop in a less regular way and perish, as a rule, at the time of the blastula formation, probably on account of irregular (multipolar?) mitosis. A shorter exposure than fifteen minutes at this temperature is, as a rule, inadequate to protect the eggs from disintegrating during the first segmentation. As was to be expected from the author's former experiments, the optimal time of exposure of the eggs to the hypertonic solution varies, if the time of exposure to the alkaline solution varies.

Unfertilized eggs of *Arbacia* were put into a mixture of 50 cc. $m/2$ (NaCl + KCl + $CaCl_2$) + 0.3 cc. $\frac{N}{10}$ NH_4OH . One part of the eggs was transferred to the neutral hypertonic solution after ten minutes, and the rest after thirty minutes. After different intervals some of the eggs were transferred to normal sea water. The result is indicated in the following table. Temperature 23°C.

(a) *Eggs in 0.3 cc. $\frac{N}{10}$ NH_4OH for ten minutes and subsequently in the neutral hypertonic solution for*

16 minutes: The eggs begin to segment, but disintegrate. No larvae formed

24 minutes: *Many eggs develop into larvae which rise to the surface*

32 minutes: *Few eggs develop into larvae. The majority disintegrate*

45 minutes: *All form membranes and begin to develop, but disintegrate*

60 minutes: *Like the preceding lot*

(b) *Eggs in 0.3 cc. $\frac{N}{16}$ NH_4OH for thirty minutes and subsequently in the neutral hypertonic solution for*

10 minutes: *About 10 per cent of the eggs form membranes and develop into perfect larvae*

15 minutes: *Practically all the eggs develop into swimming larvae, many of which are perfect. Numerous larvae rise to the surface*

23 minutes: *Very few eggs develop into larvae; the majority of the eggs undergo cytolysis*

45 minutes: *All the eggs undergo cytolysis*

It is, therefore, obvious that the eggs that had been in 50 cc. m/2 ($NaCl + KCl + CaCl_2$) + 0.3 cc. $\frac{N}{16}$ NH_4OH for ten minutes developed best when exposed to the hypertonic solution for twenty-four minutes, while an exposure of sixteen minutes was too short and one of thirty-two minutes too long. The eggs that had been in the same alkaline solution three times as long (thirty minutes), developed best when put for fifteen minutes into the neutral hypertonic solution, while ten minutes were not quite sufficient and twenty-three minutes too long.

These observations throw a light on the experiment mentioned in the previous paragraph. In this experiment the eggs were put into an NH_4OH solution containing KCN for forty-five minutes. The latter retarded the oxidizing effect of the NH_4OH and therefore had the same effect as if the eggs had been put for a shorter period into an NH_4OH solution free from KCN. We have seen, however, that a shorter exposure of the eggs to a solution of NH_4OH requires a longer exposure than fifteen minutes to the hypertonic solution if we wish to cause the development of the eggs into larvae. The eggs did not develop in this experiment because the exposure of fifteen minutes to the hypertonic solution was in this case too short.

This idea was put to a test. The unfertilized eggs of *Arbacia* were put for forty-two minutes into a solution of 50 cc. m/2 ($NaCl + KCl + CaCl_2$) + 0.3 cc. $\frac{N}{16}$ NH_4OH + 5 drops 0.1 per cent KCN. They were then transferred directly into the

neutral hypertonic solution, 50 cc. $m/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 8 cc. $2\frac{1}{2} m$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$). Part of the eggs were transferred after fifteen, twenty-five and thirty-three minutes to normal sea water. Of the eggs that remained only fifteen minutes in the hypertonic solution all perished. About 50 per cent of those that had been in the hypertonic solution twenty-five minutes developed into larvae, and a still greater part of those that had been in the hypertonic solution for thirty-three minutes developed. A large number of these larvae rose to the surface. If the eggs had been in the NH_4OH solution without KCN an exposure of fifteen minutes to the hypertonic solution would have been sufficient.

It should also be remembered that the writer had shown long ago that the action of the hypertonic solution requires also the presence of free oxygen and is delayed through the addition of KCN. When the eggs are put into the alkaline solution containing KCN for forty minutes they will not at once lose all the KCN or HCN when put into the hypertonic solution. This may be an additional reason for the necessity of keeping them longer than fifteen minutes in the hypertonic solution after a treatment with a KCN solution.

8. *Effect of the concentration of NH_4OH*

In all the experiments mentioned thus far the concentration NH_4OH used was $3/5000 N$ ($0.3 \frac{N}{10} \text{NH}_4\text{OH}$) to 50 cc. $m/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$), since this concentration was found to be very satisfactory for the production of good larvae. It was desirable to get an idea of the limits of the concentrations in which the NH_4OH can be used. To 50 cc. $m/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) were added, 0.05, 0.1, 0.2, 0.4, 0.8 cc. $\frac{N}{10} \text{NH}_4\text{OH}$ and unfertilized eggs were put into these solutions. The eggs remained in the solutions forty minutes and were then transferred into the above mentioned neutral hypertonic solutions. They remained in the hypertonic solutions for fifteen minutes and were then transferred to normal sea water. The results are indicated in the following table:

Amount of NH_4OH used:

0.05 cc. $\frac{N}{10} \text{NH}_4\text{OH}$	Two larvae found. Practically all the eggs unaltered and normal. No membranes
0.10 cc. $\frac{N}{10} \text{NH}_4\text{OH}$	Very few larvae. About half of the eggs unaltered, the rest cytolyzed
0.20 cc. $\frac{N}{10} \text{NH}_4\text{OH}$	Few larvae. Practically all the eggs cytolyzed
0.40 cc. $\frac{N}{10} \text{NH}_4\text{OH}$	A large number of larvae, part of which rise to the surface
0.80 cc. $\frac{N}{10} \text{NH}_4\text{OH}$	Very few larvae; the rest of the eggs practically all cytolyzed

These results are easily intelligible in the light of the previously described experiments. The addition of 0.05 cc. $\frac{N}{10} \text{NH}_4\text{OH}$ to 50 cc. $m/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) does not affect the eggs in forty minutes, nor does an exposure to the hypertonic solution for fifteen minutes. Practically all of the eggs, therefore, remain normal. 0.10 cc. $\frac{N}{10} \text{NH}_4\text{OH}$ is able to affect a number of eggs in forty minutes, but the exposure of fifteen minutes in the hypertonic solution is too short (see previous paragraph.) Therefore, the affected eggs perish. They might have developed had they been exposed a little longer to the hypertonic solution. 0.04 cc. $\frac{N}{10} \text{NH}_4\text{OH}$ is satisfactory for an exposure of forty minutes to the alkaline solution and of fifteen minutes to the hypertonic treatment. This series, therefore, yields a good crop of larvae, although it is not the optimum. 0.8 cc. $\frac{N}{10} \text{NH}_4\text{OH}$ is too high a concentration, for it injures the eggs and only a few survive.

9. *The individual variation of the eggs*

All of these as well as our previous experiments bring out the fact that the individual eggs vary a little in their reaction to the same solution. We are inclined to ascribe this result chiefly to a difference in the permeability of the individual eggs for bases, since it is not to be expected that the surface films of the individual eggs are exactly alike. Another source of variation seems to lie in the unequal distribution of the eggs in the solution, or at the bottom of the dish, whereby the chances for the equal diffusion of alkali or oxygen into the egg are diminished.

10. Application of the method to the eggs of other forms

The method of treating the unfertilized eggs with NH_4OH was also tried on the eggs of *Nereis* and of *Chaetopterus*. The eggs of the latter form suffer in the treatment but a small number were caused to segment. The eggs of *Nereis*, however, could be caused to segment and develop almost normally with this treatment. The method was not varied sufficiently to warrant us in giving details.

SUMMARY OF RESULTS

1. The experiments show that the weak base NH_4OH is much more efficient for the causation of artificial parthenogenesis in *Arbacia* than the strong bases KOH , NaOH , and tetraaethylammoniumhydroxide. This fact corresponds with the observation made by the writer several years ago, that the weak acids (like the mono-basic fatty acids or CO_2) are much more efficient in the same process than the strong acids. The explanation given by him for the latter case seems to hold for the former, that only that part of the acid or base which is able to diffuse into the egg brings about artificial parthenogenesis.

2. The unfertilized eggs of *Arbacia* can be caused to develop into normal larvae by putting them into a mixture of 50 cc. $\text{m}/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 0.3 cc. $\frac{8}{10}$ NH_4OH for twenty-five minutes, and afterwards into a neutral hypertonic solution, namely, 50 cc. $\text{m}/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) + 8 cc. $2\frac{1}{2}$ m ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) for fifteen minutes (at a temperature of about 22°C .) The eggs must be freed from sea water by repeated washing in a mixture of $\text{m}/2$ ($\text{NaCl} + \text{KCl} + \text{CaCl}_2$) before they are put into the alkaline solutions. This method is almost as satisfactory as the butyric acid method.

3. The eggs treated for twenty-five minutes with NH_4OH form membranes in the hypertonic solution. They can also form membranes while in the NH_4OH solution, but in that

case they must remain in the alkaline solution for a considerably longer time.

4. The effect of the NH_4OH can be inhibited or retarded by the addition of a few drops of KCN to the solution. Since our experiments indicate that only that part of the alkali can act which diffuses into the egg, and since it was shown formerly by Wasteneys and the writer that in spite of its low degree of dissociation NH_4OH is about half as effective for the increase in the rate of oxidations as KOH , the experiments suggest that in the egg of the sea urchin the oxidations are not confined to the external surface.

ON ARTIFICIAL MODIFICATION OF LIGHT
REACTIONS AND THE INFLUENCE OF
ELECTROLYTES ON PHOTOTAXIS

WOLFGANG F. EWALD

Since Loeb's early papers on animal heliotropism it has been known that in certain cases the experimenter is able to bring about changes in the orientation of animals to light by artificial means. Loeb was able to show that in marine copepods decrease of temperature and increase of concentration of the seawater made negatively phototactic animals positive, and vice versa: that increase of temperature and decrease of concentration made positive animals negative. Similar effects of temperature were noted in *Polygordius* larvae. Strassburger ('78), Massart, Holmes and Mast found the opposite effects in swarm-spores, Protozoa and a species of *Ranatra*. Later on Loeb supplemented his communications by the discovery, that freshwater *Gammarus*, *Daphnia* and *Volvox* could be made strongly positive by adding acids, especially CO_2 , to the water. The same clear results could not, however, be obtained in marine planktonic forms. In the nauplius of *Balanus perforatus* Loeb could observe a strong influence on phototaxis only by exposing the animals to very strong or very weak light. Strong light made positive animals negative, weak light made negative animals positive. The ultra-violet rays proved to be specially effective in making positive animals negative. In fact, their effect was stronger than that of all other rays combined. Rothert, in experimenting on the effect of alcohol, ether and chloroform on free swimming plants, found the light reactions of *Gonium* and *Pandorina* to be inhibited by some of these narcotics in a certain dosation, the power of locomotion remaining unaffected.

According to the investigations of Tappeiner and Hertel a similar modification of light reactions is brought about by cer-

tain dyes, especially by eosine. Hertel showed, that *Paramecia* and *Rotatoria* can be killed by light of certain wave-lengths, provided that by artificial staining their protoplasm is put in a position to absorb the light rays acting upon it. Ultra-violet light, however, obtained from the spectrum of magnesium-sparks, had a deadly effect also on unstained animals and was thereby shown to be absorbed by protoplasm under all circumstances. Green light, for instance, would attain the same result only, after eosine was added to the water containing the animals, in a dilution of 1:1200. This effect of certain dyes, because of its similarity to the process of color-sensitisation in photography, has been defined as 'sensitisation' of the protoplasm. According to Hertel, it is also possible to make the retractor-muscle of the syphon in *Sipunculus* directly sensitive to light by staining it with eosine 1:3000, while the ventral cord in the same species is stated to be sensitive to light without staining, through natural pigmentation.

Starting from these experiments I tried to obtain modifications of the light reactions in various marine animals by artificial means. I must confess that my expectation to find changes in the sensibility of a given species to colored rays of not excessive intensity by staining the animals, was not fulfilled. Nevertheless, I hope that the results obtained in the other experiments mentioned may justify publication.

My experiments were carried on in a spacious dark room of the Naples Zoölogical Institute from February to August 1911. I wish to express my special obligations to the Prussian Ministry of Education for the use of the table and to Dr. Burian, Dr. Bauer and Dr. Cerutti for their help and advice during the course of my work.

After some weeks of unsuccessful research the nauplii of *Balanus perforatus*, on which Groom and Loeb had experimented more than twenty years ago, proved to be the most satisfactory objects. These authors have thoroughly investigated and described the characteristic behavior of the nauplii. After hatching, the larvae show strong positive phototaxis. After being exposed to light for some time they first begin to oscillate between

the positive and negative orientation and then finally reverse their reaction, becoming negative with a velocity proportionate to the light intensity. If the light is very weak, the reversion does not occur at all. As the authors saw positive animals swim from direct sunlight into diffused daylight and negative animals take the opposite course, provided only that they were following the direction of the light rays, they concluded, that, in accordance with Loeb's theory of phototaxis, not light intensity, but the direction of the light rays, was the determining factor in orientation.¹ Moreover they did not succeed in finding any influence of sudden changes of intensity on the behavior of the nauplii. They concluded from experiments with red and blue glass, that the short wave-lengths were of greater effect than the long ones. Finally, they tried the effect of changes in temperature and salt-concentration, but without apparent results. Hess, in the course of investigation on the sense of sight in invertebrate animals, arrived at the conclusion, that they all showed the maximum of stimulation in the green and yellow-green parts of the spectrum, not in the blue and violet, as was to be expected by the older experiments with color-screens. This caused Loeb to reinvestigate the question, together with Maxwell. They experimented on the Californian *Balanus* and *Volvox* following the method of Hess. After placing a cuvette containing the animals, in the light of a spectrum, they found the largest gathering in the green. Hess simultaneously obtained similar results with *Balanus* at Naples.

I first examined the behavior of the nauplii in white light of a 50 c. p. electric lamp. They were seen to become negative much more rapidly on the first day after hatching, than on the following. From day to day their sensitiveness to light slowly decreased. Brought from the dark into light they all show a characteristic reaction, sinking to the bottom more or less quickly, according to the intensity of illumination and collecting there

¹ On the evidence offered this conclusion was since shown to have been drawn without cogent reason (Hess, Ewald) and the antithesis of 'intensity' and 'direction' not to be justified in this form (Ewald, Mast) though the underlying idea was a fruitful one and a step forward.

on the side facing the lamp. If the lamp is attached at a level with the animals, outside the glass containing them, they mostly remain near the bottom and but a small column of nauplii is seen along the side up to the surface. Not before the lamp is lifted considerably above the surface level does the aggregation of nauplii near the surface increase. If one now inserts a smoked glass between lamp and glass vessel, all the animals instantaneously rise towards the surface, but some of them will sink again after some time. By inserting a second smoked glass the same effect may be attained a second time, causing practically all animals to collect near the surface. If one now takes away one of the smoked glass panes, all nauplii will begin rising again for just a moment, but soon commence sinking. One observes a column of animals moving down fairly rapidly and finally stopping; gradually they begin reascending. *Obviously the nauplii of Balanus show a typical reaction to changes of intensity of illumination*, just as I was able to demonstrate it for Cladocera and Copepods. Increase of illumination (within certain limits) causes first slight acceleration, then inhibition of locomotion, making the animal sink. Decrease of illumination causes acceleration of locomotion, making the animal move toward the source of light. The same absolute light intensity will cause the 'negative reflex' when following a weaker illumination and the 'positive reflex' when following a stronger one. This shows the nauplii of *Balanus* to adapt themselves to different light intensities, provided these intensities remain unchanged for a sufficient time. If one brings the source of light vertically above the glass vessel, one does not generally succeed in observing the sinking movement after increase of illumination. The animals collect near the surface and their locomotion, directed vertically upwards, is sufficient to keep them there even after a decrease in the energy of locomotion. With the light coming from the side the locomotion is directed horizontally, and even small changes in the rate of locomotion will result in a conspicuous change of position.

I now tried to find out which wave lengths of the spectrum had the strongest influence on the reactions to changes of intensity of illumination described above. Positively phototactic animals

were distributed in eight glass tubes of 12 mm. diameter, arranged side by side in a small stand. A spectrum obtained from an arc lamp by means of a carbon bisulphide prism, was thrown obliquely from above on the row of glasses. Before beginning the experiment I made the animals, who were collected near the surface, adapt themselves to weak light reflected from the ceiling. As soon as the arc lamp was switched on, the animals began to sink in the green and yellow-green, after that in the blue-green, blue and yellow and finally in the violet and the red. The lower border of the part of the tubes filled with animals formed a blunt cone having its lowest point in the green and yellow-green. The same experiment could also be made in the reverse way. I made the animals adapt themselves to the different spectrum colors. When a strong white light was switched on at some distance from the side of the glasses, the animals in the green part of the spectrum would sink last, as they were adapted to the strongest light, those in the red and violet first.

By another experiment it can be shown, that the same rays that bring about the strong reactions to changes of the intensity of illumination have also the strongest orienting power. If a narrow cuvette with parallel sides, filled with larvae, is exposed to the spectrum, the long axis being cut at right angles by the direction of the rays, the major part of the animals accumulates in the yellow-green and green. One can observe the animals swimming toward this part from all sides, leaving the rest of the glass nearly free. In the yellow-green they gradually sink to the bottom and collect there in large numbers near the front pane. The negative animals are found in the violet and red near the rear pane, while the green part remains free of negative animals. These experiments, frequently repeated, confirm the results obtained by Hess, who found the maximum of reaction to light in a great number of invertebrates to occur in the green and yellow-green parts of the spectrum. It is important to keep in mind, that the same rays have the strongest effect on the reactions to changes of intensity of illumination described above. I find it necessary to state in parenthesis that the ani-

mals in being oriented by the light rays follow a line strictly defined by the parallelogram of forces. An animal swimming on one side of a trough covered by the light of a spectrum, say, in the violet, will be directed both by the violet rays coming from the source of light and by the blue, green and yellow-green light dispersed by the front pane of the trough, as can be seen with the naked eye. The animal may consequently be observed to direct its course between the two, arriving at the front pane, say, in the blue. It now continues to work along against the pane, but directed obliquely against the green part, till it comes to rest in the green light itself and is now oriented chiefly by the direct light. Even a very slight excess of stimulation on one side by the dispersed green rays will suffice to bring animals ultimately into the green. Loeb is therefore perfectly justified in assuming that the phototactic animal moves in the direction of the light rays and statements to the contrary made by different authors since the beginning of experiments on animal tropisms, lastly by Hess² as recently as 1911, must be based either on insufficient observation or on inaccurate reasoning. It is necessary to come to a plain understanding on this question after so many years of experiments.

Having ascertained these facts it was important to know whether the green rays possessing the strongest effect on phototactic motor reflexes would also be most important for the process of making positive animals negative. This question cannot by any means be answered in the affirmative *a priori*, as previous authors have tacitly done. If it is probable that the

² Hess's assumption that phototactic animals are not forced to move along the line of the light rays but choose their way to the field of strongest illumination—at right angles to the direction of the light rays if necessary—is due to his overlooking the facts mentioned above and to a faulty interpretation of the tropism theory. The tropism theory does not assume the animals to move towards 'the' source of light, unless there is really one source of light only. If light can strike the animal from several points, even if the excess of light on one side be very slight, it follows a course defined by the parallelogram of forces, as has been pointed out by Loeb from the very beginning. It is Loeb's merit, to have pointed out the purely machine-like and stereotyped character of phototactic reactions which differ from orientation in higher vertebrates by the very fact that there never can be any 'choice' in the direction of locomotion.

reactions to changes of intensity described above and shown to be instantaneous and very easily reversible, are effected through the medium of the eyes, this is not certain for the relatively slow non-adaptive process of negativation. Should this process be brought about by photic stimulation of the eye, we would have to assume either a second slower, non-adaptive photochemical process running alongside of the other, or else summatory action of the quick and adaptive process, leading to a new and stronger effect. These conclusions seem cogent, after the adaptive character of the reactions to changes of intensity of illumination has been recognized.

A fact which may at first sight speak against the photoreceptors as mediators of the process of negativation is the discovery made by Loeb, that ultra-violet light of a mercury lamp makes the *Balanus*-larvae negative at a quicker rate, than sunlight, especially if the latter is deprived of its ultra-violet rays by means of a glass plate. The rays of shortest wave-length would thus have a stronger negativating influence than all the other rays put together. This maximum would not coincide with that found for orientation. On the other hand, it is possible that in the case of the ultra-violet rays we are dealing with a special case influenced by the deleterious effect of these rays on the entire protoplasm; that ultra-violet light acts directly on the chemical processes of metabolism and stands apart in its effect. It is shown by the following experiment, that ultra-violet light has a strongly deleterious effect also on the *Balanus*-larvae.

Two blackened watch glasses containing animals were put in diffused daylight or sunlight. One was covered with a strong clean glass plate, the other was left uncovered. After a few minutes in sunlight or one to two hours in strong diffused light the nauplii in the uncovered dish were killed. Those in the covered dish lived for hours. I have often repeated this experiment. Every time a simple glass plate, which did not weaken the visible rays ostensibly sufficed to retard the harmful effect of strong light on the nauplii, showing thereby that the effect

was due to the ultra-violet rays. The glass plate had also a visibly retarding effect on the negativating process.

Returning to the effect of light on the process of negativation, I will first give a description of the visible phenomena connected with this process. The animals normally collect near the surface on the side of the vessel nearest to the light ('positive pole' of the vessel) provided the rays strike the vessel obliquely or from the side. If the light intensity is not inframinimal for negativation, the animals begin after some time to sink to the bottom and to collect there likewise at the positive pole. It is not before a considerable time that the second phase of the process begins, the nauplii reversing their orientation and, in consequence, collecting round the negative pole. According to the light intensity the first phase will last for a longer or shorter span of time.

To decide the effect of the different wave lengths of visible light on the process of negativation I proceeded by two different methods. First I used an arrangement similar to that described above for testing the effect of different wave lengths on the phototactic reflexes and orientation. The animals, equally divided in thirteen small tubes, were brought into the strong primary spectrum of a Rowland grating. Sunlight, projected into the dark room by means of a heliostat, was used for illumination. When the glass tubes were brought from the dark into the light of the spectrum, one could again observe the animals sinking most strongly in the green, less so in the blue, still less in the yellow, while they remained close to the surface in the red and the violet. This difference was observed to persist, if the light rays struck the glasses about horizontally, this being a case of permanent regulation of the position by light intensity. If the nauplii were not too old—younger than forty-eight hours—they would begin to become negative after some time, first in the green, shortly after in the violet. Later on the animals in the blue-green and blue followed, finally those in the yellow. In the red part only a very slight negativating effect could be observed. One glass served as a control and was placed outside the spectrum. Here the animals would not become negative. As soon as the animals became

negative, they were removed from the glasses with a pipette. As a consequence, the tubes in the violet and the green light were first emptied, all nauplii having become negative; the blue and the yellow tubes followed much later, and the red would take a very long time.

Similar results were obtained with color-filters. The same glass tubes which had been disposed in the spectrum were put into wider glasses containing a colored liquid. The glass tubes were corked and entirely surrounded by the liquid. The filter-liquids consisted for

Violet of methyl-violet (area transmitted from 400-470 μ and from 610-700 μ)

Blue of copperacetate + methyl-violet with addition of ammonia (area transmitted from 445-485 μ)

Green of copperacetate + potassium monochromate with addition of ammonia (area transmitted from 480-560 μ)

Red of lithium carmine (area transmitted from 610-700 μ)

The layer of colored liquid surrounding the glasses was nowhere less than 12 mm. deep. The transmitted areas were tested with a Zeiss spectroscope and as far as possible the extinction of all the areas was made equal by means of the Engelmann 'Microspectralphotometer,' that is to say: each area was made to transmit the same fraction of the colored light of equal wavelengths existing in the solar spectrum by regulating the saturation of the colored solution.

As in the previous experiments, the different position of the animals in each color after distribution in the different glasses was very noticeable; as usual the nauplii were nearest the bottom in the green, nearest the surface in red and violet. If the sensitiveness of the larvae stood in a favorable proportion to the intensity of illumination, it was frequently observed, that they became negative in the violet and green only and remained positive for several hours in all other colors. If I then brought the glasses into full sunlight, the animals became negative also in the blue and finally in the red light. Brought back into diffused daylight they became positive in the reverse order. The experiments with color-filters are, however, not as conclusive as the others, owing to the impossibility of separating with

sufficient exactitude the effect of the extinction of the monochromatic light from the effect of its wave length. In fact the effect varied, especially in the green, according to the opacity of the solution.

We may state in conclusion, that *two maxima of negativating effect were found inside the visible part of the spectrum: one in the green and one in the violet, the minimum being in the red.* If we were to show the negativating effect of light rays by a curve, we should have to begin with a maximum in the ultra-violet slowly falling towards the blue, rising again to the yellow-green and falling steadily towards the minimum in the red. The nature of the curve makes it probable, that we have to deal with at least two interacting effects, one of which may operate by the medium of the eyes (maximum in the green) while the other acts on some body substances through the cuticle directly (maximum in the ultra-violet).

I cannot, however, refrain from mentioning the fact, that this result was not always attained. Especially during experiments in which the pure, strong and broad spectrum of the Rowland grating was replaced by the considerably smaller, weaker and less pure spectrum of a carbon bisulphide prism, the effect in the green was invariably so small, that I stated a minimum instead of a maximum in this part. The negativating influence of the yellow and even the red rays was sometimes stronger than that of the green ones, whereas the effect on the motor reflexes was also in this case strongest in the green.

To conclude, I will mention a few experiments on the deleterious effect of concentrated monochromatic light. If the animals contained in the color-filter glasses described above were exposed for about two hours to full sunlight, they were killed first in the violet, later in the green and the blue and not at all in the red. To the eye the red solution seemed most transparent, the violet nearly opaque. The permeability to ultra-violet rays was probably equally small in all glasses.

I shall now proceed to describe a series of experiments carried out in order to determine the effect of variations of the temperature and the chemical composition of the water on the light

reactions of *Balanus*-larvae. Before describing these experiments, it should be mentioned that my results were always controlled by comparison with animals, caught at the same pole of the same vessel as the animals experimented on, but kept in pure seawater of normal temperature. This is important, as the animals change their reaction when exposed to light without any other influence being brought to bear on them, and as it is therefore possible only to determine the influence of the agent to be tested by comparison with normal animals under equal conditions of illumination (and temperature). I will mention first the effect of changes in temperature.

I found without exception, that increase of temperature made positive animals negative and negative animals more negative, and that decrease of temperature made negative animals positive and positive animals more positive. I never noted any uncertainty in this effect of temperature. The amount of the change in temperature necessary for the reversing of the reaction to light depends on the age and state of the larvae. The relation between the inclination to positive or negative reaction, the quotient $\frac{p}{n}$ indicating the degree of neutralisation of two antagonistic processes causing positive or negative reaction, constitutes what is called the 'Lichtstimmung' of the organism. The factor of 'Lichtstimmung' must be considered in all observations on the effect of stimulating agents. If, in our case, the nauplii are approaching the negative condition owing to the effect of illumination, a slighter increase in light intensity will effect the change of reaction than if they had just been exposed to light. Young animals are more easily influenced than old ones. A change in temperature of about 5 or 6°C., however, always has the desired effect. At about + 5°C. the nauplii fall into cold rigor; they will stand being treated to more than 30°C. As an increase of about 1°C. will under certain circumstances—especially if the larvae are newly hatched—suffice to make them negative, it is most important to maintain a constant temperature during all experiments on phototaxis.

I will next discuss the influence of the salts of the seawater on phototaxis. In this connection I desire to express my thanks to Dr. O. Meyerhof, who gave me many valuable hints. Acting on his advice I used 0.65 molecular solutions which corresponded best to the concentrations found in the Naples Aquarium water at the time. I made up solutions of chemically pure sodium chloride, potassium chloride, magnesium chloride, magnesium sulphate and calcium chloride. The magnesium and calcium chloride were tested by titration. The effects of the different salts were studied firstly by adding them to pure isotonic sodium chloride solution, secondly by adding them to natural seawater and finally by eliminating successively each component salt from artificial seawater made up of the five components mentioned, with the addition of the necessary amount of alkali. The artificial seawater had the following composition:

	cc.
0.65 mol. NaCl.....	100.0
0.65 mol. KCl.....	2.2
0.65 mol. MgCl ₂	6.6
0.65 mol. MgSO ₄	4.2
0.65 mol. CaCl ₂	2.0
1.00 mol. NaHCO ₃	0.25
0.10 mol. NaOH.....	0.2

The amount of alkali is that found by Warburg at the Naples Institute by comparison with natural seawater. The animals kept well in this artificial seawater, if not quite as long as in natural seawater. There was no effect on phototaxis on transferring the nauplii into artificial seawater. That is: the time elapsing until the reverse of orientation under the influence of a certain source of light was neither shorter nor longer than in control animals kept in natural seawater. The artificial seawater was therefore in its influence on phototaxis equivalent to natural seawater.

When the animals were transferred into pure isotonic NaCl solution, negative larvae became positive, positive larvae more positive. Moreover, positive animals would not become negative again, even under the influence of full sunlight, including the ultra-violet rays. They died after a short time through

the action of the ultra-violet rays, sinking down on the positive side of the glass. They had apparently lost the possibility of negative orientation. The same effect could also be obtained by adding to the NaCl solution pure natural seawater in the proportion of 1:1; in this case the animals were less affected by the solution, while in pure NaCl they sometimes died after a quarter or a half-hour. Added to natural seawater in small doses, NaCl solution accelerates the positive and retards the negative reaction at a degree depending on the value of the quo-

tient $\frac{P}{n}$ (Lichtstimmung). Correspondingly, the threshold concentration required to bring about positive reaction in negative animals is different. Leaving NaCl out of artificial seawater has an effect contrary to that obtained by adding it to pure seawater. It increases the negative and diminishes the positive reaction. The best solution proved to be one containing about two-thirds of the ordinary amount of NaCl. Lower concentrations were harmful. The result obtained pointed to the probability, that there were other salts in the solution antagonistic to NaCl, whose effect predominated when NaCl was diminished.

Potassium chloride had an effect similar to, but considerably weaker than that exhibited by sodium chloride. Pure isotonic KCl solution killed the animals instantly and if added to natural seawater in proportions above 1:150 proved to be poisonous also. If the concentration is slightly weaker than 1:150, positive reaction is increased, negative reaction diminished, but I never saw large quantities of animals become instantly positive, as they did after a sufficient addition of isotonic NaCl. KCl does not change the effect of pure NaCl solution if added in the proportion prevailing in natural seawater, nor does its omission from artificial seawater cause any alteration of the reaction.

Calcium chloride belongs to the same group as the two first mentioned salts. Pure 0.65 molecular solution has a toxic effect. If added to natural seawater in the proportion of 1:15 it inhibits negative reaction in a very short time, both in negative and in positive animals about to become negative. But it has the singular secondary effect of apparently paralyzing the larvae. The

rhythm of the locomotor movements is retarded and the orientation to light is at length totally abolished, causing the animals to swim about unoriented. It would therefore be hardly appropriate to attribute a positivating influence to CaCl_2 . In a long standing tube with the source of light high above it, when normal animals would gather near the surface, animals in the CaCl_2 solution are equally distributed throughout the entire length of the tube; the reaction to changes of intensity is considerably diminished or disappears entirely. Nevertheless, the animals live indefinitely in such a solution and swim about continuously. With CaCl_2 also there was no effect when it was added to isotonic NaCl or omitted from artificial seawater.

As in the two last mentioned salts, so in magnesium chloride, the pure solution was toxic. I may recall the fact that in pure NaCl solution the nauplii would not become negative. If, however, MgCl_2 or MgSO_4 was added in the ordinary proportion of artificial seawater to pure NaCl solution, the retarded negative reaction would be called forth instantly, provided the light was strong enough. There is no appreciable difference in the rate of negativation under the influence of a given source of light between larvae in natural seawater and in the mixture of sodium and magnesium solution. *Apparently magnesium has the opposite or antagonistic effect to sodium.* It was now the question, whether magnesium has in itself a negativating effect, analogous to increase in temperature, or whether it merely compensates the effect of sodium (potassium) if present in a definite proportion to these salts. This question is answered by experiments, in which magnesium chloride or sulphate was added to natural seawater. In this case there is no negativating effect whatever, even with the strongest concentration the larvae could permanently stand (1:25). We are therefore justified in assuming that magnesium acts only as a compensation to the positivating influence of sodium but possesses no negativating influence. This is confirmed by the experiments with artificial seawater without magnesium. If the solution contains no magnesium, the effect is similar to that of pure sodium chloride, but the animals would keep no better than in pure NaCl solution.

When natural seawater is added to the artificial magnesium-free seawater in a proportion of 3:1, the animals would live in this solution for some hours without becoming negative, save in a few exceptional cases. Decrease of magnesium has therefore the same effect as increase of sodium or potassium. In other words, *for a normal production of light reactions it is necessary to have the correct proportion of sodium (potassium) on one side and magnesium on the other.* In my experiments magnesium chloride proved slightly more effective than the sulphate.

Lastly, among the constituents of seawater I have to mention the alkali content. With the concentration prevailing in natural seawater no effect could be detected. When all alkali was left out of artificial seawater or added to pure NaCl solution, the reaction of the nauplii was not changed. A stronger concentration of OH-ions, however, (about 2 cc. $\frac{N}{15}$ NaOH in 100 cc. of seawater) had a visible negativating influence; ammonia was still more effective (about 1 cc. $\frac{N}{10}$ NH_3 in 100 cc. of seawater), due according to the experiments on sea-urchin eggs, to its permeating more quickly into the protoplasm. The reaction of the seawater had in both cases become strongly alkaline to neutral red. Both alkalies in the concentration mentioned would quickly make positive animals negative and prevent negative animals from becoming positive, even in very weak light.

I believed it to be of interest, with reference to the papers by Loeb mentioned above, to see whether acids would have the opposite effect. Contrary to the negative results that author obtained in marine forms of America, I found that the mineral acids HCl, H_2SO_4 and HNO_3 had a positivating effect, but that the effective concentration had very narrow limits. Only such concentration would prove successful, as gave neutral red a slight pink hue, natural seawater giving a reddish yellow color. Slightly higher concentrations killed the animals, lower ones had no effect. In acetic acid and CO_2 I saw no positivating influence nor was it very strong in the mineral acids mentioned.

Lack of oxygen has a very strong effect on phototaxis. If nauplii are put in seawater which has been evacuated for a

few minutes by means of a filter pump (at about 300 mm. of mercury pressure) they become positive immediately and remain so as long as they are left in the water. Transferred into fresh water they become negative again with equal velocity. This is one of the strongest and most striking reactions. Traces of metal acted in the reverse sense. If traces of copper (Cu , concentration = 2.10^{-6}) are added to the seawater, all positive animals soon become negative and remain so. This effect is in accordance with the experiments on oxydation in the sea-urchin egg, where traces of metal also acted in the same sense as alkali and increase in temperature. I did not, however, repeat this experiment more than three or four times with different animals.

I found no marked positivating or negativating effect with narcotics, of which alcohol, ether and chloroform were tried, though the tendency was rather in the former direction. They would, however, disorient the animals, like calcium, causing them not to assemble at the poles of the vessel but to swim about freely in all directions.

Experiments with potassium cyanide, strychnine, oxygen and hydrogen peroxyde met with no success. Finally, I made experiments with changes in concentration. The result was the same observed by Loeb with Copepods: increase in concentration had a positivating effect, decrease a negativating one. My best hypertonic solution contained 1 gram of pure crystalline NaCl in 100 cc. of seawater. The effect was very strong. To exclude the possibility, that the higher concentration of Na ions was responsible for this result, I made a hypertonic solution of magnesium chloride which could in no way be suspected of a specific positivating salt action. The positivating effect was less marked but sufficiently evident. I am therefore justified in concluding, that hypertonicity alone has a positivating influence, though in the first case it may have been seconded by the effect of the increase in Nations. Increase in concentration has the opposite effect. Seawater mixed with fresh water in the proportion of 7:1 quickly makes positive animals negative and prevents negative ones from becoming positive.

Unlike the Californian form used by Loeb my nauplii showed no lack of precision in their response to changes of concentration.

To sum up, we may repeat the names of all the agents experimented with, classed in three groups. The first contains those with a positivating effect, including sodium, potassium, acids, deoxygenated seawater, hypertonic seawater and decrease of temperature. The second contains those with a negativating effect, including certain visible and invisible light rays, alkali, traces of metal, hypotonic solution and increase of temperature. The third contains narcotics, causing the animals to lose their sensitiveness to light and including calcium, alcohol, ether and chloroform.

I will conclude the account of my work on *Balanus nauplii* by a number of experiments made to test the influence of various dyes on phototaxis. The larvae were transferred for some time into solutions of stains and then exposed to light of different wave lengths either in these solutions or in pure seawater. I tried methylene blue, eosine, erythrosine, Bismarck brown, methyl green, neutral red and orange. The effect of staining on the light reactions was ascertained by comparison with unstained animals, special care being taken to keep both stained and unstained animals under equal conditions. The water was always taken out of the same aquarium for both sets of animals. The only variable was the addition of the dye.

I soon observed that animals which had been stained in Bismarck brown or methylene blue became negative more quickly than unstained larvae when exposed to white light. It was sufficient for the animals to remain for one or two hours in a solution with just a shade of brown or blue color, to obtain this result and the same effect was reached when the animals were put into the color solution and exposed to light at once. With eosine the effect was very slight and not always observable and with the rest of the dyes no effect was seen at all.

I then exposed animals stained brown or blue to strong artificial light made monochromatic by color solutions. I used a solution of potassium monochromate for the yellow and a solu-

tion of copper acetate with addition of methyl green and lithium carmine for the blue filter. In both colors the animals stained with Bismarek brown became negative first, those stained with methylene blue second and the unstained ones last. I was therefore not able to state that the complementary colors chiefly absorbed by the dye (yellow for the methylene blue and blue for the Bismarek brown) had a stronger effect than those of the same color. This is, however, what ought to have been the case, if I had succeeded in producing a 'sensitisation.' Moreover, the difference in the velocity of negatization between stained and unstained animals was not considerable, the negatizing effect of the dyes being very small in comparison to most of the agents mentioned in this paper. The effect of heat was not, however, to be made responsible for the result; special measurements showed that the differences never exceeded 0.4 to 0.5 of a degree (C.), the water being warmer sometimes in the colored and sometimes in the uncolored water. The poisonous effect of the dyes made it impossible to use higher concentrations. All these observations indicated the probability that the effect of methylene blue and Bismarek brown had nothing to do with their color but with their containing some chemical agent, which would make the animals negative in small concentrations and kill them in stronger ones. It is well known that methylene blue is poisonous for living protoplasm, especially in the light, and the same is true, according to Straub, for eosine, which is said to form a hypothetical poisonous eosine peroxide under the influence of light. In my experiments all the dyes mentioned proved to be toxic even in the dark in concentrations slightly higher than those used for my purposes. To obtain a final answer to the question at issue I made use of the reaction to changes of intensity of illumination, mentioned in the beginning of this paper. Three glass tubes with brown, blue and unstained nauplii were made to adapt themselves to weak light coming from above. After some time, the strong monochromatic light was turned on, the source of light being at a level with the surface of the water in the tubes. The nauplii sank every time with equal velocity in all three tubes, whether I

turned on the yellow or the blue light. I concluded that there is no sensitisation of the protoplasm of eye or body of the nauplii by the dyes used and that their negativating influence is probably due to a non-photochemical effect on the protoplasm.

Concluding from the information gained by my experiments, on the behavior of free *Balanus* larvae under normal conditions, it may be supposed that they react very similarly to the other planktonic forms investigated by the present author. After hatching, the larvae swim towards the surface, the strong increase of light causing them to sink down again very soon by inhibition of their locomotion. Their movements will consist in a continuous alternation of sinking and rising ('periodical locomotion,' Ewald '10) caused by successive inhibition and stimulation, without ever necessitating the taking place of negative reaction. This reaction probably constitutes an artificial product of the laboratory. The 'periodical locomotion' as described in the paper referred to above, causes the animals to maintain themselves in an area of equal illumination throughout the day, taking them gradually up in the evening and down in the morning. In the evening, decrease of illumination will slowly shift the position where inhibition due to prolonged upward locomotion takes place, nearer and nearer the surface, while the reverse is the case for the morning. It is thus unnecessary to assume that the animals constantly change between positive and negative reaction, as was supposed by Loeb. The eminent usefulness of this mechanism is shown by the experiments demonstrating the strong deleterious effect of the light rays of short wave lengths on the nauplii.

SUMMARY

I. Effects of light

1. The nauplii of *Balanus perforatus* show the same reactions to changes of intensity of locomotion, as that found by the author in Cladocera and Copepods. Increase of illumination causes inhibition of locomotion, preceded by a slight acceleration; the result is a sinking. Decrease of illumination causes

acceleration of locomotion. Within limits, the absolute intensity of illumination does not affect these reactions.

2. It was found that light of different wave lengths influenced these reactions in a different way. Green and yellow-green have the strongest effect; blue-green, blue, yellow, violet and red follow in the order named.

3. The same order of efficiency is found, when the orienting power of different parts of the spectrum is tested. The positive animals collect in the green on the side near the light, the negative animals in the red and violet on the far side.

4. The velocity with which positive animals become negative is also different for different parts of the spectrum, but in a different order. *Balanus* larvae became negative most quickly in the violet and in the green, less quickly in the blue and the yellow and hardly at all in the red.

5. The ultra-violet rays have the most strongly deleterious effect on the larvae. The violet rays follow next, then the green and the blue, while the red rays of the intensities tested had no harmful effect.

II. Effects of temperature

6. Increase of temperature made positive animals negative and negative animals more strongly negative; decrease of temperature made negative animals positive and positive animals more strongly positive.

III. Effects of the salts of the seawater in isotonic solutions

7. Isotonic sodium chloride solution, pure or added to natural seawater, makes negative animals positive and positive animals more positive. If sufficiently in excess of the other salts, it inhibits negative reaction entirely.

8. Isotonic potassium chloride solution, added to natural seawater, acts in the same direction, though less effectively.

9. Isotonic calcium chloride solution, added to natural seawater, makes the larvae lose their power of reaction to light stimuli, causing them to swim about at random without negative or positive orientation.

10. Magnesium chloride or sulphate solution acts as an antagonist to sodium. Added in the proportion prevailing in natural seawater to pure NaCl solution, $MgCl_2$ brings about the negative reaction which is suspended in pure NaCl solution. There is no difference in response to photic stimuli between larvae in the sodium magnesium mixture and in pure seawater. For a normal production of light reactions it is necessary to have the correct proportion of sodium on one side and magnesium on the other.

IV. Other chemical effects

11. Sodium hydrate or ammonia above a certain concentration had a strong negativating effect.

12. The mineral acids HCl, H_2SO_4 and HNO_3 in a certain concentration had the opposite effect. Acetic acid and carbonic acid had no effect.

13. Lack of oxygen (brought about by evacuating the seawater) had a very strong positivating effect.

14. Traces of copper had a negativating effect.

15. Alcohol, chloroform and ether caused the animals to lose their reactions to light.

V. Effects of changes in concentration

16. Hypertonic solutions of NaCl or $MgCl_2$ had a strong positivating effect, hypotonic solution of NaCl had an equally obvious negativating effect.

VI. Effect of stains

17. Staining nauplii with Bismarck brown or methylene blue had a weak negativating influence, due, however, not to a specific color effect ('sensitisation') but to certain chemical contents of the stain. Other stains tried had no effect whatever.

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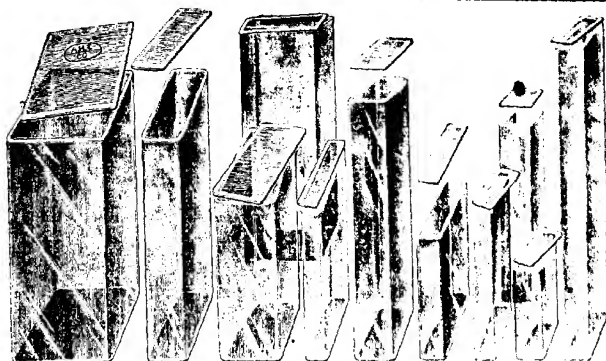
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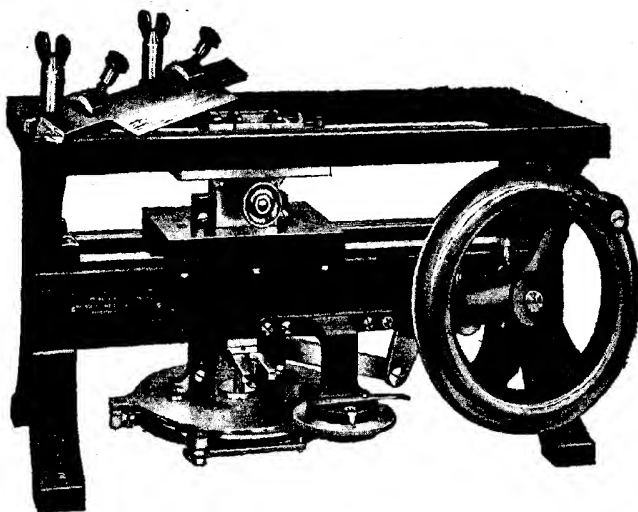
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